EXPERIMENTAL STUDIES OF LATENT Q FEVER INFECTIONS

by

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INTRODUCTION

The ability of some pathogens to induce latent infections in susceptible hosts has been recognized as an epidemiological problem since early in the Nineteenth century, especially when the reactivation of such infections was instrumental in the spread of the manifested diseases through various populations (Ford, 1939; Dubos, 1958). Coxiella burnetii, the causal agent of the rickettsial disease Q fever is among the known pathogens which exhibit this latent infection capability (Stoker, 1957). The organism has been shown to persist in animal tissue for long periods of time after experimental infection (Sidwell, 1961), and its persistence has been determined by repeated isolation of the rickettsiae from tissue of certain wild animal species in nature (Sidwell, 1962). Because of this latency property, many animals may be regarded as potential carriers of C. burnetii.

The mechanism of activation of latent or dormant infections is still unclear, although it is known that certain changes in a host's physiological condition can cause such a reactivation to occur (Andrewes, 1958). Among the factors which may accomplish this alteration of host condition are irradiation, administration of certain hormones, and pregnancy.

The objectives of the present investigation were (1) to further study the persistence of C. burnetii in wild and laboratory animal tissues; (2) to attempt to correlate persistence of rickettsiae in such latently

infected animals with contamination of the surrounding environment; (3) to investigate the effects of whole body x-irradiation, cortisone injections, and pregnancy and/or parturition with respect to the ability of each to reactivate latent C. burnetii infections; (4) to show a relationship between application of stress and spread of infection from Q fever infected animals to uninfected cage mates and (5) to study the Q fever antibody response in infected animals subjected to x-irradiation, cortisone, pregnancy, or a second infection with C. burnetii.

Deer mice, common rodents native to the Great Salt Lake Desert of Utah, guinea pigs and white mice were employed in these investigations. The antibody responses were studied by means of Phase I and II complement fixation (CF) and Phase I capillary tube agglutination (CTA) tests.

LITERATURE REVIEW

The experimental work which is presented in this study is essentially an extension of preparatory work reported earlier (Sidwell, 1961). In the previous study much of the pertinent literature was reviewed concerning Q fever, including history, natural and experimental infection in wild animals, antibody response, rickettsiae in animal tissues, rickettsial strains, and diagnostic tests. Therefore, this review will present information directly pertaining to the present study, namely, latent infections, mode of transmission of C. burnetii and results of work by others employing x-irradiation and cortisone to modify rickettsial infections. In addition, since the nature of the study directly involves mechanisms of resistance, this subject will also be discussed as pertaining to Q fever.

I. LATENT INFECTIONS

A. Definitions and History

A latent infection is defined here as an inapparent infection which may be chronic, and in which a certain agent-host equilibrium is established. The term "latency" was agreed upon for viruses at a symposium on latency and masking in viral and rickettsial infections (Walker et al., 1958) and as defined above it will be employed in this study for the disease Q fever. "Reactivation" of a latent infection is used to mean any alteration in the persistence of the pathogen in tissue in such a way that other tissue or tissues of the host animal subsequently become infected. In this manner the disease agent may become infectious for another host.

The fact that a pathogen can be latent, or dormant, showing no apparent manifestations of its presence, has long been known. Probably one of the earliest recognitions of such infections occurred in Ireland in the 1840's. A potato blight was noted only during unusual weather and growth conditions, although the causal fungus, Phytophotora infestans, was normally carried by most potato plants at all times (Dubos, 1958). In the early 1890's Koch showed by isolating Cholera vibrio from feces that healthy human beings could play a role in the transmission of cholera (Ford, 1939). Pasteur showed that fowl cholera bacilli were harbored in experimentally infected guinea pigs for long periods of time in localized abscesses, although the health of the animals was apparently not seriously impaired (Ford, 1939). Prior to the reports by Koch and Pastuer, several bacteriologists had observed that virulent organisms persisted in the body after recovery from clinical attacks of a disease (Roux and Yersin, 1890; Loeffler, 1890; Gutmann, 1892), but the significance of these observations relative to the spread of diseases was overlooked (Stanier, et al., 1957). The discoveries of Koch and Pasteur thus started a reinvestigation of carriers of latent organisms.

Koch (1903) first made the distinction between "temporary carriers", convalescents who discharged organisms in fecal material or urine for short periods only, and "chronic carriers" who had completely recovered from disease and yet harbored the causal organisms in some part of the gastrointestinal or urinary tract and excreted them in feces or urine. His conclusions were based on work with typhoid fever. Koch's term "chronic carrier" will be considered analogous

to the "carrier of latent organisms" used in the present study. Other early work showing latent infections included investigations on dysentery (Conradi, 1903), paratyphoid fever (Prigge and Sachs-Muke, 1910), plague (Gaffky, 1908) and epidemic cerebrospinal meningitis (Kutscher, 1906). Typhoid fever, first studied in this respect by Koch, has been quite extensively investigated, the later results in most cases merely confirming the original findings (Gregg, 1908; Irwin and Houston, 1909; Garbat, 1922). The subject of typhoid fever and typhoid and other carriers was first extensively reviewed by Ford (1939).

Although studies of latency and carrier state were initiated relatively early in the modern history of bacteriology, more evidences of the extensiveness of this phenomena have been gathered only in the past two decades. The main conclusion of these investigations show unequivocally that pathogenic agents to which a population is susceptible frequently become established in the tissues of a large percentage of persons, yet few or no signs of the actual clinical disease may be detected (Dubos, 1958).

Most of the progress in the field of latent infections has developed from studies of viral and rickettsial infections. Examples of the former are the several types of adenoviruses found in the tonsils and adenoids of healthy persons (Rowe et al., 1953), the many plant viruses which can be isolated from healthy plants (Bawden, 1958), herpes simplex virus infection in man, which often is periodically activated (Buddingh et al., 1953), and the tumor viruses such as Rous sarcoma virus (Bryan, 1958).

Since this study is concerned with Q fever, a rickettsial disease, it will be this type of disease that will be the principal area of review. Several major rickettsial diseases have been reported as capable of becoming latent and reoccurring at a later period of time. These are Q fever, Brill-Zinsser disease, trench fever, Rocky Mountain spotted fever and psittacosis. (The classification of the psittacosis agent as rickettsiae is arbitrary and will be discussed further in Part F of this section.)

B. Q Fever

Marmion et al. (1954) reviewed the subject of Q fever infections in human beings, emphasizing the concept of latent Q fever infections. Q fever was first observed to be a latent infection when relapses occurred following apparent recovery in human patients (Beck et al., 1949; Andrews and Marmion, 1959; Robson and Shimin, 1959; Bertrand and Roux, 1955). Coxiella burnetii was isolated from the blood of several patients showing a relapse of the disease or, in one instance, at autopsy from the valves of the heart of an endocarditis patient (Smith and Evans, 1960). Syrucek et al. (1958) reported the isolation of infectious quantities of the rickettsiae from the placenta of three women who were in labor and from uterine curettments from another in whom pregnancy had been interrupted because of rubeola. All the women had past histories of Q fever infection, the period between infection and delivery ranging from 2 years and 8 months to 3 years and 2 months. In all cases delivery resulted in the birth of a healthy infant and the post partum period was also free of complications. Earlier results of this

same nature were reported by Babudieri (1953) who isolated C. burnetii from a human placenta. The fact that rickettsiae were recovered from the above sources indicates a possible method of contamination of the surroundings. Q fever infections in newborn infants have also been reported (Gaburro and DelCampo, 1956) in which one child of triplets died of the disease. Sera from the two surviving infants had positive CF titers against C. burnetii. Both parents were also seropositive, indicating a possible passage of organism from parent to offspring.

Stoker (1957) discussed the problem of latent infections with viruses and rickettsiae, particularly stressing the fact that among the rickettsiae a C. burnetii infection is notorious for its ability to be reactivated after long periods of dormancy. This reactivation of the infection has been observed especially in cattle, sheep, and goats during parturition (Kilchsperger and Weisman, 1949; Luoto and Huebner, 1950; Welsh et al., 1951; Abinanti et al., 1953a; Stoker et al., 1955). The observed reactivation of the infection among livestock was the apparent cause of spread of the rickettsiae resulting in many human cases of the disease.

C. burnetii has also shown latency characteristics in experimentally infected domestic fowl (Gallus gallus domesticus) (Sobeslavsky, 1957). In this experiment hens were infected subcutaneously, intraperitoneally, or intranasally. Although no signs of illness were observed in most of the fowl, organisms were excreted in the feces between the 14th and 42nd day after infection. Attempts to reactivate infection in some of these birds by injections of cortisone were unsuccessful. Eggs laid by the hens 19 and 42 days after infection were shown to contain C. burnetii and rickettsiae could be demonstrated from animals hatched

from some of the eggs between 2 and 55 days after hatching (Sobeslavsky and Syrucek, 1959). A transovarial transmission by this species of bird was thus established.

The nature of latent infections with the Q fever rickettsiae has recently been investigated. Kordova (1960) inoculated guinea pigs, hamsters, mice and chicks intraperitoneally with filtrates of suspensions of C. burnetii passed through membranes of 58 and 40 mu mean porosity. Most animals showed no clinical, serological or microscopic signs of infection. However, serial passage of tissue homogenates from these filtrate-injected animals through 2-week-old guinea pigs or embryonated eggs revealed infectious quantities of the organism. The author considered that these results pointed to the passage of specific genetic material of C. burnetii through the filters, this material being capable of persisting in a latent form in apparently healthy animals. Under suitable conditions it could then develop into typical "mature" rickettsiae. He assumed that the filtrable particles represented an intracellular stage of the developmental cycle of the organism, a stage which may correspond to the vegetative genome postulated by Lwoff (1958) as occurring in latent viral infections. Earlier studies by Kordova (1959a, 1959b) showed that the filterable particles of C. burnetii were too small to be visible in the ordinary light microscope.

Phase variations of the Q fever rickettsiae (Stoker, 1953; Stoker and Fiset, 1956; Fiset, 1957) may play a role in determining the nature of latent infections. This variation is a change in the antigenic structure of the organism after passage through eggs. Egg-adapted

C. burnetii is considered to be in Phase II while animal-adapted rickettsiae are in Phase I. The agent, in Phase I when recovered from mammals, was described as being more virulent than the Phase II form (Brezina, 1958). Phase I antibody has been shown to be more protective than Phase II antibody (Abinanti and Marmion, 1957). Fiset (1957) suggested that both types of antibody are produced by the animal, but one is ineffective, due to a polysaccharide on the surface of the Phase I rickettsia which prevents the main protein antigen of the organism from combining with its antibody. Phase II organisms inoculated into female ticks were demonstrated to be transferred transovarially to the tick larvae. When reisolated from the larvae the rickettsiae were found to be in the Phase I form. The reisolated organisms, in contrast to their activity in mammals, multiplied poorly in yolk sacs and had a low virulence for guinea pigs (Brezina and Rehacek, 1961). An explanation of this low virulence in Phase I form may be that the organisms of low virulence were mutants selected by the passages through the ticks.

It is possible that the organism exists in the animal body in a state of low virulence or in a suppressed state, able to infect and perhaps destroy a small group of cells locally, but unable to establish or re-establish a general or widespread infection. This ability to initiate a general infection may be blocked by specific immunity of the host, as indicated by the presence of antibody, or by genetic or species resistance to the infection. However, as speculated by Kordova (1960), the organism may not have the ability to initiate or establish a general infection because it is in an "immature" stage of development when latent,

requiring an especially susceptible cell to be activated into a mature, virulent form. From the experimental work cited, the observed latency property may be due to suppressive action of the host on the infection, or may be an alteration in the development of the Q fever rickettsiae.

C. Brill-Zinsser Disease

Brill-Zinsser disease, often called recrudescent typhus, is a relapse of epidemic louse-borne typhus fever in relatively mild form years after the primary attack. The causal agent is Rickettsia prowazeki. The disease was first noted by Brill (1910) when it occurred sporadically in persons in New York City and in other areas. The causal rickettsiae were shown by cross immunity studies to be the same as that causing epidemic typhus (Anderson and Goldberger, 1912).

The disease was later shown to take place chiefly among refugees to this country and case records of many indicated it to be a recrudescence of an epidemic typhus infection which had occurred years earlier (Zinsser, 1934). Zinsser postulated that the typhus rickettsiae persisted during the latent interval somewhere in the tissues of the human subject. This postulate was confirmed in part by Price (1955), when he isolated virulent R. prowazeki from the lymph nodes of two supposedly healthy persons who had been in an environment believed to be free of epidemic typhus prior to recovery of their organisms. Both subjects had antibody of low titer in their sera, possibly indicating a previous typhus infection. Price (1955) was also able to activate a latent epidemic typhus infection in cynomolgus monkeys by cortisone injection. The factors which precipitate Brill-Zinsser disease are not known, although it is postulated that

the balance between the immune mechanism and the parasite is in some manner disturbed, resulting in a recrudescence (Snyder, 1959).

A significant point concerning Brill-Zinsser disease is that following recrudescence of the disease, human body lice become infected from the human patient (Murray et al., 1950; Murray and Snyder, 1951) thus resulting in a new epidemic of typhus fever. This has apparently occurred in Yugoslavia (Murray and Gaon, 1958).

D. Trench Fever

Trench fever, caused by Rickettsia quintana, was unknown until 1915, but during World War I it afflicted a great number of men. Among the symptoms was an attack of fever, which in about half the infected persons reoccurred from 3 to 8 times (Swift, 1919-20). Some patients apparently had a latent infection, with lice biting them and becoming infected as long as 300 to 443 days after onset of the illness (Strong et al., 1918). The causal rickettsiae have been isolated from persons having a history of the disease as long as 1 and 2 years after initial illness (Weyer, 1954; Mohr, 1954). However, in these cases the possibility of reinfection was not excluded.

E. Rocky Mountain Spotted Fever

Rocky Mountain spotted fever is a tick-borne disease of the Americas, caused by Rickettsia rickettsii. This disease agent can be classified with those causing latent infections primarily due to its longevity in ticks and its alteration into a virulent organism by blood meals or incubation within the gut of ticks (Spencer and Parker, 1923; Price, 1953b, 1954). The organism has also been reported to

to persist in certain mammalian tissue for as long as a year (Parker et al., 1954), although no reactivation of infection was demonstrated.

F. Psittacosis

The psittacosis agent has been considered a rickettsia by many authorities (Moshkovsky, 1945; Philip et al., 1953; Breed et al., 1957; Giroud et al., 1955). Because of this, the organism will be discussed with the rickettsiae. Latent psittacosis infections first came to the attention of investigators when supposedly healthy birds died of the disease when subjected to various types of stress while undergoing shipping. The birds apparently acquired the infection as nestlings (Meyer and Eddie, 1933; 1934; Meyer, 1942). The agent has been reported to persist for at least 7 months in mice (Meyer and Eddie, 1933; Bedson, 1938). The principle organs in which the latent infections occurred in these animals were spleen, liver and kidney (Meyer and Eddie, 1933). Chick embryos experimentally infected 0 to 18 days after incubation still carried the agent when hatched into chicks and the agent persited up to 22 days in these chicks (Davis and Vogel, 1949). Meyer (1942) was able to isolate the agent from the ovaries and egg yolks in the oviducts of parakeets and suggested that infection may be transmitted from latently infected adults transovarily through the egg. This ability of the psittacosis agent to pass from the parent to the developing embryo was also demonstrated by Storz (1961) in guinea pigs. The young guinea pigs that contracted the infection *in utero* were observed for 4 weeks. They were apparently unaffected by the infection and developed normally.

Seemingly uninfected sheep have been found to excrete the psittacosis agent in their feces for long periods of time. Upon pregnancy the disease becomes acute, often causing abortion (Dungworth and Cordy, 1962; Storz, 1962).

Latent psittacosis infections in man were probably first described by Gerlach (1936) when the agent was isolated from sputum samples of five persons showing no symptoms of the disease. Haagen and Mauer (1938) and Doerr (1944) reported similar findings and speculated that patients convalescing from clinical psittacosis may serve as healthy carriers and shed the agent for varying lengths of time without a renewed exposure to a natural reservoir. Such a carrier was found by Meyer and Eddie (1951), who isolated the organism from sputum of a man known to have had psittacosis 10 years previously. This man had complained of various symptoms continually since the original infection, including a hacking cough with copious amounts of sputum. Surprisingly, no persons coming in contact with the patient apparently became infected.

Austin (1957) employed various types of stress in an attempt to reactivate latent psittacosis infections in experimentally infected mice. Some of the stressor agents used were epinephrine, insulin, triple typhoid vaccine, dinitrophenol, "histamine acid phosphate", hyaluronidase, mucin and cortisone injections. The effects of x-irradiation, pregnancy and deficient diets were also investigated. Only daily cortisone injections of 5 mg subcutaneously for two days or a diet deficient in nicotinamide were reported to alter the latent infection. This was determined on the basis of deaths occurring in the animals within 10 days and isolation of the psittacosis agent from the dead mice. The num-

ber of organisms in the spleen increased ten-fold in the 10 days after cortisone injection. No change could be observed in the CF antibody level.

II. MECHANISMS OF RESISTANCE TO COXIELLA BURNETII

Since the Q fever organism differs markedly from the other, more classical rickettsial species, this discussion concerning mechanisms of resistance will deal with that organism only.

One of the primary defense mechanisms, phagocytosis, has been demonstrated only to a small extent. Victor et al. (1952) reported that the phagocytosis of C. burnetii was governed by all the usual factors affecting in vitro phagocytosis of a bacterium such as Brucella suis. These factors included period of contact between phagocyte and organism, temperature, agitation, number of organisms present, type of phagocytes, and anticoagulant used. In these investigations polymorphonuclear neutrophils were employed to demonstrate phagocytosis. The principle purpose of the experiments was an attempt to demonstrate a rise of opsonin titer in the serum of infected patients and guinea pigs. A positive opsonic index was clearly demonstrated and opsonin titers rose in some animals much higher than complement-fixing antibodies. The report failed to mention, however, whether the rickettsiae were observed to be digested in the neutrophils. This would be important since Burnett and Freeman (1937) showed that Q fever rickettsiae proliferated in splenic and renal histiocytes of mice and guinea pigs. Whittick (1950) also has reported finding large quantities of the rickettsiae in the histiocytes of the lung, spleen, and testes of a human who died of

the infection. Rychlo and Pospisil (1960) described lymph node infections with C. burnetii, in which it was observed that large, vacuolated reticuloendothelial cells containing colonies of the rickettsiae eventually burst, liberating the organisms.

In cases of fatal Q fever, in which death was due to a diffuse pneumonia, histiological examinations have been quite revealing in regard to phagocytic activity in Q fever infections (Lillie et al., 1941; Perrin, 1949; Whittick, 1950). Although the gross appearance of the consolidated lungs closely resembled that seen in pneumonococcal lobar pneumonia, the histological aspect closely resembled that seen in psittacosis and some viral pneumonias. The histology varied with the stage of the pneumonic process at which the tissue was examined. In areas of red hepatization affected alveoli were filled with a small quantity of polymorphonuclear neutrophils and mononuclear cells in approximately equal numbers, with lesser amounts of lymphocytes and plasma cells. In areas of gray hepatization, the inter-alveolar septae were considerable thickened by lymphocytes, plasma cells, large mononuclear cells and neutrophils. Alveolar exudate consisted largely of swollen mononuclear cells, with some neutrophils and red cells present.

Specific antibody apparently plays a significant role in host resistance to C. burnetii infection. Fever was reported to be suppressed when serum from an immune animal (bandicoot) was mixed with the rickettsial suspension prior to inoculation into guinea pigs (Derrick et al., 1939). Other investigators (Burnet and Freeman, 1937; 1940; Bengston, 1941a) reported finding a fever-suppressing property of immune

sera from white mice, guinea pigs and rabbits. Q fever immune sera have been shown to diminish the multiplication of the organisms when mixed with the rickettsiae and injected into mice. This was determined by showing a reduction in the number of rickettsiae found in smears of the mouse spleen (Burnet and Freeman, 1938b, 1939).

Agglutinating antibody to C. burnetii was first demonstrated by Burnet and Freeman (1937), and has since been employed as a diagnostic tool by many investigators. Complement fixing antibody to C. burnetii was first reported by Bengston (1941b), and its detection is also now being widely used as a serologic test aid in the diagnosis of the disease.

Phase variation (discussed previously under I-B) apparently is important in immune responses of the animal to C. burnetii. Phase I antibody is more protective, as determined by measuring the neutralizing capacity of the sera obtained from white mice (Abinanti and Marmion, 1957). Investigations have been carried out studying the possible correlation of Phase I and II CF Q fever antibody responses to rickettsial persistence in tissue of various animal species (Sidwell, 1961). It was shown that Ord kangaroo rats (Dipodomys ordii), which produced very little detectable Phase I CF or capillary tube agglutination (CTA) antibody, contained C. burnetii in some tissue for nearly one year after initial infection. Phase II CF antibody was detected in comparatively high titers in these animals. Conversely, the Q fever rickettsiae also persisted for long periods of time in tissue of desert wood rats (Neotoma lepida), which formed relatively high titers of detectable Phase II and Phase I CF antibody but only low or moderate titers of Phase I CTA antibody. This may

indicate the more protective activity of Phase I antibody, particularly of the agglutinating type.

Infection of experimental animals or man with C. burnetii usually produces an effective immunity against reinfection. This was first demonstrated by Derrick (1937) and Burnet and Freeman (1937) in guinea pigs and white mice. Subsequent investigators have employed formalin-killed organisms in attempts to induce an effective and lasting immunity (Smadel et al., 1948; Zubkova et al., 1956a, 1956b; Kulagin et al., 1958b; Benenson, 1959). Varying claims have been made by these workers as to the efficacy of the vaccines produced. In a series of experiments Siegert et al. (1953) tested the immunogenic value of large doses of C. burnetii vaccine prepared from yolk sac cultures which had been killed by ultraviolet irradiation after purification by ether extraction. The CF and agglutination antibody response of guinea pigs inoculated with this material varied in relation to the size and number of doses administered. According to this publication, no worthwhile immunity against Q fever infection could be produced. The authors suggested that reports of enhanced resistance by other earlier investigators may have resulted from incompletely killed rickettsiae contained in the preparations. Studies by the author (Sidwell, 1961) comparing the Q fever antibody responses in various wild rodents, as well as laboratory animals, correlated well with the studies of Siegert and his group. Phase I and II CF and CTA antibody were detected only in low titers in animals inoculated with killed rickettsiae. Animals inoculated with living organisms produced much higher detectable antibody titers. The strain of C. burnetii employed apparently has a great deal to do with the inducement of this antibody response

(Fiset, 1959).

A protective mechanism of some importance in animals is the interference phenomenon. Clinical observation from as early as the 16th century has shown modifying effects when a second infection has been superimposed on the first. An alteration of the second infection, or both first and second infections, occurred (Schlesinger, 1959). This modification in the course of the diseases in mixed infections has been termed the "interference phenomenon". The phenomenon has been observed principally in viral-viral infections (Henle, 1950; Schlesinger, 1959), viral-bacterial infections (Dalldorf et al., 1947), bacterial-bacterial infections (Nyka, 1955), rickettsial-rickettsial infections (Price, 1953a), and other systems. The phenomenon was reported in Q fever infections by Mika et al. (1954) and Victor et al. (1955). It was found that the course of both brucellosis and Q fever was much milder in mixed infections in guinea pigs if the animals were infected with Brucella suis prior to infection with C. burnetii. The severity of Q fever infections was much milder using both lethal and sub-lethal doses of C. burnetii. This was found when either or both agents were given by parenteral or respiratory routes, providing the bacterial infection was induced 11 days or more prior to the introduction of the rickettsiae. Other work showed that passive immunization with single or multiple injections of Brucella antisera failed to alter the severity of Q fever infection. Active immunization of the test animals against the bacteria prior to secondary infection also failed to yield protection (Mika et al., 1958). But Brucella endotoxin when administered 11 days or more prior to injection of rickettsiae gave protection (Mika et al., 1959).

Coxiella burnetii when given prior to the bacteria was found to give protection in guinea pigs against Pasteurella pestis infection. Other rickettsiae also demonstrated this ability and to a greater extent than the Q fever organism (Owen and Larson, 1956).

The exact mechanism of the interference phenomenon is not known, although some of the observed results indicate some aspects of the mechanisms. Specific antibody does not seem to be a factor, since serological tests have failed to show any cross-reacting or similar antibodies (Mika et al., 1958). Passive immunization using immune sera did not modify the imposed infections. Previous immunization appeared to have no effect. The immune phagocyte apparently does not play an active part, since in the Br. suis-C. burnetii infection prior immunization failed to modify the results. The "activated phagocyte" may be implicated in interference since protection was shown only where an active infection was in progress or where a dose of toxin or dead cells had been previously injected.

There are undoubtedly numerous host mechanisms of resistance to Q fever infection. Although no specific work has been reported, it is assumed that the usual factors involved in natural immunity would be applicable to C. burnetii infections. These factors include the skin, buccal and nasal cavities, stomach, intestines, lungs, eyes, genital-urinary tract, the inflammatory response and the lymphatic and circulatory systems, all of which involve the numerous cellular and humoral factors. It is apparent from the previous experimental work cited that C. burnetii is able to penetrate and oversome some of these natural mechanisms of defense quite readily. Since the disease is manifested so frequently as an interstitial pneumonia, it is apparent

that the pharyngeal cavity, mucous membranes, the lungs and the cellular mechanisms involved in the lungs are inadequate in the resistance of the host. The disease often becomes systemic following a rickettsemia, which indicates the lymphatic and cirulatory systems, together with the concomitant cellular and humoral factors, are also relatively impotent in checking the spread of the organism. Experimental studies have shown, however, that a host's acquired immunity to the agent is very effective, as previously discussed. This acquired resistance probably involves both cellular (increased digestive capacity of the immune phagocytic cells) and humoral (specific antibodies) factors, which probably act in unison.

Genetics has been implicated as a definite factor in host resistance to Q fever infection since susceptibility to infection with the disease agent varies with animal species (Lennette, 1959; Sidwell, 1961; Horsfall and Ferris, 1962). Other factors such as nutrition, fatigue, age, mental state, environment (temperature, humidity, etc.) and general social conditions probably also play an indirect role in a host's resistance to C. burnetii, but to date experimental evidence is lacking on this aspect. Certain chemicals and drugs are known to depress resistance, usually through depression of phagocytosis or other physiological functions, but relatively little has been reported concerning resistance to Q fever infection. Hormonal affects will be discussed in section V of this literature review.

III. TRANSMISSION OF COXIELLA BURNETII

A major difference between Q fever and the other rickettsial diseases stems from their routes of transmission. Instead of being

transmitted ordinarily by the bite of an infected arthropod, C. burnetii usually infects man by the respiratory route through inhalation of rickettsiae-laden aerosols. Lower animals probably also become infected in this manner, although other methods of transmission of the disease agent are known, and will be discussed subsequently.

A. Arthropod Vectors

Since one of the original isolations of C. burnetii was from a tick (Davis and Cox, 1938), this form of vector has been quite extensively investigated. Although at least 33 species of ticks have been found naturally infected with the rickettsiae (Sidwell, 1962) the acquisition of Q fever in this way is apparently very rare. Only a few case reports can be cited in which infection was related to tick bites (McGurl and Williams, 1948; De Prada et al., 1950; Babudieri, 1951; Derrick, 1953). Other arthropods have also been implicated because of the recovery of the disease agent from them in nature or as a result of demonstrating their ability to transmit the organism. These arthropods include lice, mites, fleas (Sidwell, 1962; Horsfall and Ferris, 1962) and possibly bedbugs (Daiter, 1960). Although few human cases have been reported implicating arthropod vectors, they should not be ruled out as a means of transmission of C. burnetii in nature.

B. Ingestion

Milk has been implicated as a mode of transmission of C. burnetii. Of 300 cases in the Los Angeles area, one-third of thoseinfected used raw milk, although less than 5 per cent of the total milk distributed in the area was unpastuerized (Beck et al., 1949). Q fever infections were ten times as frequent among raw

milk drinkers in populations not exposed to livestock (Bell et al., 1950). Huebner et al. (1948, 1949) isolated C. burnetii from raw milk samples of more than half of the dairies in the Los Angeles area. In contrast to the above implication, man has been shown to be only moderately susceptible to Q fever infection by the gastrointestinal route (Fonseca et al., 1949), thus the role of milk in transmission of the disease to man may be minor. There have been many reports, also, of pasturized milk drinkers who were infected (Lennette and Clark, 1951; Clark et al., 1951a, 1951b). It would appear that infective milk could serve as an excellent means of infecting suckling offspring.

Infection may also occur from ingestion of other food or water contaminated with C. burnetii. Many animal tissues have been reported to contain C. burnetii, the rickettsiae persisting for periods greater than one year (Burnet and Freeman, 1938b; Davis and Cox, 1938; Lennette et al., 1952; Sidwell, 1961). Silich (1957) demonstrated that meat can remain infective for as long as 150 days when frozen. The rickettsial agent also will survive in ordinary tap water for over 150 days (Kulagin et al., 1958a).

C. Aerosol Exposure

The means of transmission described thus far can be implicated only to a small extent so far as is known from available literature. The primary mode of transmission for Q fever, as previously pointed out, is by aerosol exposure. Coxiella burnetii is noted for its ability to survive when subjected to adverse conditions. Thus temperatures as high as 60°C will not readily inactivate the rickettsiae (Ransom and Huebner, 1951; Kirberger, 1951) nor will ultraviolet

irradiation (Kirberger, 1951) or desiccation (Smadel, 1952). Tick feces may retain infective C. burnetii for 586 days at room temperature (Philip, 1948) and dried guinea pig blood containing the organism remains infectious at least 182 days (Parker et al., 1949). The rickettsiae were reported to survive at least 109 days in cell-free media held at 28°C (Cox, 1939) and in tap water as discussed in III-B. Because of this resistance property C. burnetii may survive on contaminated objects for extensive periods of time and be transported over long distances or by indirect paths to susceptible hosts.

Dust may consequently play an important role in the spread of Q fever. A number of reports have been published in which C. burnetii was isolated from dust taken from livestock pens (DeLay et al., 1950; Lennette and Welsh, 1951; Vuksic et al., 1956; Welsh et al., 1958, 1959). Dust has been implicated in a number of outbreaks of the disease (Robbins et al., 1946; Clark et al., 1951b; Giroud et al., 1953). Materials such as hair, wool, hides, etc., or clothing may also become contaminated and result in a spread of the disease agent (Oliphant et al., 1949; Sigel et al., 1950; Stoker and Marmion, 1955).

The methods by which the above stated materials or objects may become contaminated are diverse. It has already been pointed out that milk may contain C. burnetii, and that placentas and birth fluids may be highly infectious after parturition in livestock and possibly other animals. Oral and nasal secretions and urine and feces from sheep were shown to contain infectious quantities of the rickettsiae (Morozzi, 1951; Lennette et al., 1952; Abinanti et al., 1953b). Similar results were also observed in studies with nasal mucous and feces from cattle (Bell et al., 1949).

Urine and feces from susliks (Yevdoshenko et al., 1961) and guinea pigs (Davis and Cox, 1938; Parker and Steinhaus, 1943) may contain C. burnetii, the organisms persisting as long as 100 days in the latter species. Urine from white mice (DeMattia et al., 1952; Ozbil, 1955) and hamsters (Bock, 1954), and feces from domestic fowls (Sobeslavsky, 1957) may also contain C. burnetii. Eggs laid by chickens infected with the Q fever rickettsiae have been shown to contain the organism (Sobeslavsky, 1957) and if broken represent another possible means of contaminating objects. Extrapolation of the cited observations can undoubtedly be made to other domestic or feral animals.

IV. EFFECTS OF IRRADIATION ON RICKETTSIAL INFECTIONS

Various types of irradiation have been extensively studied with regard to their effects upon animal systems. The primary reasons for these studies are the possible uses of radiological warfare, the increasing employment of radiation in science and industry, and the growing interest in the effects of "natural" irradiation, i. e., cosmic rays, ultraviolet rays, etc. As discussed earlier, Q fever infections have been demonstrated to be reactivated naturally. Whole body x-irradiation seemed a logical method by which such a reactivation might be induced experimentally.

In referring to effects of irradiation, the paucity of available information regarding Q fever necessitates a broadening of the review material to include other rickettsial diseases.

A. Q Fever

Burnet and Freeman (1938b) were able to increase the number of Q fever rickettsiae in the spleens of experimentally infected mice by subjecting the mice to whole body x-irradiation. The dosage given was approximately 900 r. According to the literature, the only other work

concerning the influence of irradiation upon C. burnetii infection was by Goldwasser et al. (1951), in which it was reported that whole body x-irradiation increased the severity of the infection in white mice.

B. Murine Typhus

The earliest reported studies of x-irradiation upon rickettsial infections was by Zinsser and Casteneda (1932). By intraperitoneally infecting rats whose resistance had been lowered by x-irradiation, these investigators were able to recover relatively large numbers of Rickettsia mooseri from the animals' organs. This work was carried out in an attempt to produce a vaccine containing more rickettsiae.

Greiff et al. (1953) reported that multiplication rate and quantity of R. mooseri increased in fertile eggs exposed to a single dose of x-irradiation. Irradiation was given either 48 hours after or 24 hours before inoculation. A later study by the same workers (1957a) showed that best effects were produced by irradiation given 6 days after inoculation. Beta-ray irradiation produced by tritium-labeled water inoculated into eggs had similar effects on murine typhus infections (Greiff et al., 1955, 1960). X-irradiation appeared to neutralize the rickettsiostatic effects of high incubation temperature and of streptomycin in eggs, although the effects of para-aminobenzoic acid, penicillin and chlortetracycline were not altered. It was speculated by the authors of this work that the streptomycin inactivation may be due to combination of the drug with degradation products resulting from irradiation or the presence of abnormally large amounts of substances which compete with streptomycin (Greiff et al., 1953, 1957b).

C. Epidemic Typhus

In a study similar to that which is to be reported in this thesis, Iatsimirskaia-Krontovaskia et al. (1959) investigated the possi-

bility of prolonged carriage of R. prowazeki in guinea pigs and rabbits. Whole body x-irradiation (200 to 800 r) was given to the animals 6 to 38 months after experimental infections with the rickettsiae. Attempts to isolate the organism from the blood and tissues of the irradiated animals were unsuccessful and the complement-fixing antibody titers in sera from the animals remained virtually unchanged.

Exposure of monolayer cultures of chick embryo entodermal cells to doses of 10,000 r of Co^{60} gamma radiation 8 to 18 hours before inoculation with R. prowazeki did not affect the growth of the organisms (Weiss and Dressler, 1958). Doses of 150,000 r to 300,000 r produced increasing degrees of cellular injury; this was associated with a greater release of rickettsiae into the media than occurred in controls. This release of rickettsiae took place during the first few days of infection, probably as a result of more rapid lysis of irradiated host cells.

Liu et al. (1941) reported that previously x-irradiated white mice were fatally infected with R. prowazeki inoculated intra-abdominally. Larger amounts of rickettsiae were recovered from the irradiated animals' tissues than could be obtained from unirradiated controls.

D. Rickettsialpox

Beta-or x-irradiation of embryonated eggs prior to or during infection with Rickettsia akari resulted in an effect opposite to that obtained with the other types of rickettsiae discussed previously, multiplication of the rickettsiae being inhibited (Greiff et al., 1960). The combined data of the R. mooseri and R. akari studies suggested to these investigators that host cell damage apparently took place primarily

in the nucleus, since the murine typhus organism usually grows well in the cytoplasm while the rickettsialpox agent multiplies in both the nucleus and the cytoplasm. This conclusion was previously proposed by another worker (Gray, 1956).

E. Scrub Typhus

In the only work reported involving irradiation and the scrub typhus agent, Kawamura and Shimuzu (1955) demonstrated that multiplication of R. orientalis and R. tomiyai was increased in x-irradiated embryonated eggs.

Various types of irradiation have been shown to be capable of activating latent infections and of increasing an animal's susceptibility to disease. This is chiefly due to the stress caused by the agent in the animal, resulting in an alteration of many of the physiological processes of the body to infection (Donaldson, 1954; Talmadge, 1955; Donaldson and Marcus, 1956; Perkins, 1958; Pottinger, 1961; Leone, 1962).

V. STEROID HORMONES AND RICKETTSIAL INFECTIONS

Information in available literature concerning effects of steroid hormones upon Q fever infections has been sparse. Therefore, other rickettsial diseases, in addition to Q fever, will also be discussed in this section.

A. Q Fever

Domestic fowl experimentally infected with C. burnetii excreted the rickettsiae in the feces for up to 6 weeks after infection. Attempts were made to revive the infection in two of the five infected hens with injections of cortisone, but were apparently unsuc-

cessful (Sobeslavsky, 1957).

Mika et al. (1959) studied the effects of various stressor compounds on mixed infections of Br. suis and C. burnetii. Earlier studies by this group showed that prior infections with the bacterium resulted in a resistance to usually lethal doses of the rickettsiae (Mika et al., 1954, 1958; See II of the review). Injections of suspensions of desiccated thyroid extract at the time of infection with C. burnetii caused a reversal of the previously observed resistance, guinea pigs dying within 10 days. Cortisone injections in the same study also appeared to nullify the interference phenomenon, if initiated with the bacterial injection. If the injections were started 14 days later, at the time of rickettsial injection, the resistance of the animals was maintained. The data suggested that the cortisone treatment depressed the overt inflammatory response and directly or indirectly altered the activity of the reticuloendothelial system associated with the primary bacterial infection. The degree of suppression of the generalized inflammatory reaction varied directly with the amount of cortisone employed (Mika et al., 1959).

B. Rocky Mountain Spotted Fever

Aikawa and Harrell (1953) studied the effects of injections of cortisone acetate on experimental Rocky Mountain spotted fever in the guinea pig. They reported little or no effect on the experimental rickettsiosis and mortality rate was not altered. Workman et al. (1952) used a combined treatment of cortisone and chloramphenicol in three severe human cases of the disease. An excellent response

was described, the fever period being several days shorter than cases treated with chloramphenicol alone. Cortisone dosage was 200 mg initially and two further doses of 100 mg at 6 hour intervals. Children received two-thirds of these doses.

C. Psittacosis

As discussed previously, psittacosis infections can be latent in birds and some mammals. Austin (1957) demonstrated that cortisone given subcutaneously in doses of 5 mg daily for 2 days in previously infected white mice caused a reactivation of the infection. This was indicated by death of 56% of the mice within 10 days after administration of the drug. The number of psittacosis organisms in the spleen rose one log unit in this same time. No change was reported in CF antibody level.

D. Boutonneuse Fever

Giroud et al. (1959) found that the effects of intracutaneous inoculation of Rickettsia conori in rabbits treated with cortisone varied considerably. Among the treated animals, seven of nine showed negative, weak or delayed local reactions, but deaths occurred in the remaining two rabbits within seven days. All agglutination titers to R. conori were either low or undemonstrable.

E. Scrub Typhus

Doses of cortisone ranging from 1.5 to 3 g per 45 kg given to human patients infected with scrub typhus reduced the typical fever and headache accompanying scrub typhus disease (Wisseman et al., 1954).

F. Epidemic Typhus

Whitney and Anigstein (1953) showed that adminstration of cortisone to rabbits or guinea pigs during the process of immunization with R. prowazeki antigen caused a low degree of suppression of CF antibody formation. Dosages for rabbits was 10 mg daily for 15 days, while that for guinea pigs was 25 mg daily for 11 days. Later work by Genig (1959) and by Shmeleva (1959b) indicated that guinea pigs treated with cortisone and inoculated with a virulent strain of R. prowazeki reacted to the infection with a shorter incubation period and a longer febrile period than did untreated animals. The rickettsiae apparently persisted longer in tissues and rickettsemia was intensified and prolonged in the treated animals.

G. Murine Typhus

Extensive investigations concerning effects of cortisone on experimental murine typhus have been carried out recently (Whitmire and Downs, 1954; Downs and Whitmire, 1957; Whitmire, 1957). Cortisone treatment was shown to alter the course of R. mooseri infection in mice, cotton rats, hamsters and guinea pigs. In all cases the susceptibility of the animals to lethal or infectious doses was increased. Scrotal reactions and inflammatory responses in the hamsters and guinea pigs were reportedly suppressed. Different strains of white mice showed great variation in their susceptibility to the effects of cortisone (Whitmire and Downs, 1954). Whitmire (1957) was also able to transform a latent R. mooseri infection in hamsters into an active, fatal infection by cortisone treatment, death occurring 30 days after infection. No controls (cortisone

treated but not infected) were reported in these experiments, so that the observed deaths could have been due to the cortisone alone. No change in CF antibody titer could be detected. Dosage of cortisone in these experiments was 10 mg with initial injection of rickettsiae and 5 mg in a single dose 24 days later. The same group of investigators (Downs and Whitmire, 1957) studied the effects of cortisone on immunity and the immune response in mice, cotton rats, hamsters, guinea pigs and rabbits. It was found to have no effect on the development of immunity or on the resistance to challenge with murine typhus in the mouse. The drug also had no apparent effect on immunization of rabbits with sonic-treated or whole rickettsial antigens. Cortisone did appear to cause a slower development of CF antibody in the rabbit, although by 21 days a higher level of antibody was present in the cortisone-treated animals. Insufficient data were presented to provide any information on effects of the drug on resistance of other animal species to murine typhus infection.

Shmeleva (1959a, 1959b) essentially repeated the work of Whitmire and Downs with similar results. Some additional data gave evidence that the CF antibody response in treated white rats was delayed the first two or three weeks after infection, but by 60 and 92 days the antibody level was higher than in control animals. No differences in CF antibody response to murine typhus organisms in cortisone-treated white mice could be demonstrated at 21 and 30 days after infection.

A prolongation of murine typhus rickettsemia by cortisone treatment of white rats was reported by Pollard and Wilson (1955). The rickettsemia lasted as long as 28 days in the treated animals compared

to only 15 days in untreated controls. Injections of ACTH prior to injection of toxins of R. mooseri or R. prowazeki apparently do not exert a noticeable protective effect (Kass et al., 1951). Mice treated in such a manner died approximately as rapidly as did non-treated controls.

Studies concerning cortisone or other adrenal cortical hormones on infectious diseases and immune processes have been subject to much attention in the past few years, primarily since the advent of synthetic cortisone. A number of reviews and entire books have been published on the subject (Thomas, 1953; Schwartzman, 1953; Dougherty, 1953; Rose, 1959; Brandon, 1962). The general effects of these hormones as described in the above reviews will be described in the Discussion section of this thesis, under Cortisone Treatment.

MATERIALS AND METHODS

I. ORGANISMS EMPLOYED

The California AD strain of C. burnetii was used in this study. The strain was originally isolated from the milk of infected dairy cattle in Los Angeles, California (Huebner et al., 1948). It was received as yolk sac slurry from Fort Detrick, Maryland, where it had been subjected to over 25 egg passages. Upon receipt, the material was distributed into glass ampules, sealed and stored on dry ice. The 50% infective dose (ID_{50}) for this strain of C. burnetii was determined for guinea pigs in a previous study by employing the following criteria: fever response; Phase II CF antibody response; and tissue infectivity. The ID_{50} dose was found to be a 10^{-9} dilution of the rickettsiae-yolk sac slurry (Sidwell, 1961). The ID_{50} of the slurry was redetermined for the current studies and again found to be a 10^{-9} dilution.

II. EXPERIMENTAL ANIMALS

Animals employed were guinea pigs (Cavia cobaya), Swiss strain of white mice (Mus musculus), and laboratory reared deer mice (Peromyscus maniculatus sonoriensis LeConte). Guinea pigs weighed 400-500 g at the time of initial inoculation and 700-900 g at time of treatment. The white mice had been weaned 3-4 weeks prior to time of inoculation. All deer mice were young adults, 7-9 generations removed from wild parent stock. Approximately equal numbers of both sexes were employed for most experiments. All guinea pigs were pre-bled from the orbital sinus and their sera tested for Q fever antibody prior to use. Random samples of white mice and deer mice were sacrificed,

bled, and their sera also tested for presence of Q fever antibody before attempting any experimentation. All pretested guinea pigs and white mice had no detectable antibody, but several sera from pretested deer mice did contain low (1:8-1:16) CF titers of antibody reacting with Q fever antigen; it was assumed, however, that these titers were non-specific, having been observed in control animals in earlier work (Sidwell, 1961).

In the reactivation experiments, all animals were caged singly to avoid cross-infection. In the attempts to demonstrate the transmission after reactivation, uninfected animals were placed as cage mates with the test animals. Normal uninfected animals were housed in cages randomly spaced among cages of infected animals to serve as controls and to indicate the occurrence of possible cross-infections between cages. Normal animals were also kept in an uninfected area to serve as additional controls.

III. SEROLOGICAL TESTS

Two types of tests were employed in these studies to detect antibody to C. burnetii. These were the capillary tube agglutination (CTA), and the complement fixation (CF) test. Each of these tests were employed and standardized in an earlier investigation (Sidwell, 1961).

The CTA test was originally described by Luoto (1953, 1956). Antigen for the test was prepared from Phase I Ohio strain C. burnetii by Dr. Luoto and associates at the Rocky Mountain Laboratory. Sera to be tested were diluted with normal sterile 25 per cent bovine sera in 0.85 per cent saline to enhance agglutination (Luoto, 1956).

The CF test employed for these studies used two exact units of complement, two antigenic units of antigen, and two units of commercial hemolysin (Cappel laboratories). Complement was pooled from a small group of normal guinea pigs, sealed in glass ampules, and stored at dry ice temperature until use. Combined serum, antigen and complement were incubated overnight at 4-6°C, after which sensitized sheep red blood cells were added, and the test incubated in a 37°C water bath for one hour. Hemolysis of 50 per cent or less was considered positive. Appropriate controls were run with each test. Antigens used in the test included a Phase I CF antigen prepared from Ohio strain C. burnetii by Dr. David B. Lackman and associates at the Rocky Mountain Laboratory, and a Phase II CF antigen of Nine Mile strain C. burnetii obtained from Lederle Laboratories.

Sera from the animal species employed in these studies were of such a nature that little detectable anticomplementary (AC) reaction could be demonstrated. Therefore with the exception of starving all animals at least 24 hours before bleeding, no extra measures had to be taken to eliminate AC reactions.

IV. TISSUE INFECTION DETERMINATIONS

All sacrificed animals were examined for spleen enlargement and a variety of organs removed, depending upon the experiment. Each viscus removed was divided into two parts. One part was pooled with other parts of the same organ type. The other half of the divided tissue was placed in separate vials and held at dry ice temperature. The organ pools were ground in a mortar with sterile sand, then suspended in saline and 1.0 ml injected intraperitoneally (ip)

into one guinea pig for each pool. These tissue-injected guinea pigs employed for the determination of the presence of C. burnetii will be referred to as "indicator" guinea pigs. These indicator guinea pigs were bled 28 days following injection and their sera tested for Phase II CF antibody to C. burnetii. Antibody titers of 1:16 or greater were considered positive in these studies, as based on earlier investigations (Sidwell, 1961). When a positive tissue pool was detected, the individual tissues comprising the pool were thawed and processed individually in a manner analogous to the above. Occasional Machiavello stains on the individual tissues were prepared and examined microscopically for the presence of visable C. burnetii, particularly when abnormal organs, e.g. enlarged spleen, were observed. Pooling organs prior to use of the individual tissues aided in conserving indicator guinea pigs and was a method of confirming positive specimens.

Blood, urine and feces were tested in a similar manner to the above, with the exception that all urine and feces were first mixed with 0.5 ml penicillin (Pronapen, Pfizer, 150,000 u penicillin G Procaine and 50,000 u penicillin G potassium), held at room temperature one hour, and then injected into guinea pigs. This procedure was undertaken in an attempt to reduce death in animals due to bacterial infection. A somewhat similar technic was employed successfully by Morgan (1949).

V. X-IRRADIATION STUDIES

A. Irradiation Method; LD_{50} Determination

A Westinghouse Quadrocondex x-ray machine was used for all irradiation experiments. Radiation factors were 250 KVP, 15 ma, 1.0

mm Al and 0.5 mm Cu filters in addition to an inherent filtration of 2.5 mm Al and 0.25 mm Cu; distance from focal point to surface for all animals was 60 cm. All radiation measurements were corrected for barometric pressure.

The average dose rate as measured by a Victoreen r meter varied from 5 r to 65 r per minute depending upon point of measurement on the table. To attempt to produce a more standard field, four lead rings 20 inches in inside diameter were set in a pile upon the surface showing highest irradiation received per minute. Following several ring adjustments on the table surface, a steady irradiation rate of 42 $\pm$ 2 r per minute was obtained at all points on the surface of the field within these rings. The measurements were made prior to, during, and following irradiation of animals. All animals to be irradiated were placed on the surface within these rings. A galvanized wire screen of 1/2 inch squares was set within the rings close to the surface over the animals to keep them spread out as well as confined. This wire screen produced no detectable fluctuation in x-irradiation dose per minute in the area holding the experimental animals. Total dosages given were calculated in proportion to time exposed. White mice and deer mice were irradiated ten at a time, guinea pigs four at a time. A 21 day LD_{50} was determined in all animals using the Reed and Muench (1938) method for calculation.

B. Reactivation Study Dosages

Dosages given in the reactivation experiment were selected to be slightly less than and greater than the LD_{50}. These were termed

the low and high doses, respectively. For guinea pigs these were 100 r and 175 r (LD_{50}: 163 r), for white mice 300 r and 450 r (LD_{50}: 431 r), and for deer mice, 350 r and 625 r (LD_{50}: 588 r).

VI. CORTISONE STUDIES

A. Cortisone Employed

The cortisone used in these studies was obtained from California Corporation for Biochemical Research (Cortisone, Cat. No. 2351). This was suspended in saline at the concentration desired and kept in a near homogenous state during injection by stirring with a magnetic stirrer. An automatic syringe was used for injections.

B. Reactivation Study Dosages

Two doses of cortisone were investigated for each animal species. For white mice and deer mice these were: 0.25 mg in 0.2 ml per animal per day for 7 days (1.75 mg total, termed the low dose) and 2.5 mg in 0.2 ml per animal per day for 7 days (17.5 mg total, termed the high dose). Injections were administered subcutaneously (sc) in the nape of the neck. For guinea pigs the doses were 2 mg in 0.5 ml per animal for 7 days (14.0 mg total, termed the low dose), and 20 mg in 0.5 ml per animal per day for 7 days (140.0 mg total, termed the high dose). Injections into guinea pigs were administered sc in the inguinal region.

RESULTS

I. DETERMINATION OF AN X-IRRADIATION LD_{50} IN GUINEA PIGS, WHITE MICE AND DEER MICE

Deaths occurring among irradiated guinea pigs, white mice and deer mice within 21 days after exposure are indicated per number tested in Table 1. Guinea pigs were most susceptible to whole body x-irradiation, as measured by the number of post-exposure deaths, animals dying from levels as low as 125 r. The LD_{50} was calculated as 163 r.

White mice were more resistant than guinea pigs to irradiation, the LD_{50} being 431 r. White mice died from doses as low as 375 r.

Deer mice were the most resistant to whole body irradiation, requiring a dose of 588 r for an LD_{50} although deaths occurred in animals receiving as low as 375 r.

II. RICKETTSIAE APPEARANCE AND PERSISTENCE IN INTRAPERITONEALLY INFECTED GUINEA PIGS, WHITE MICE AND DEER MICE

An earlier study (Sidwell, 1961) had demonstrated the persistence of the California AD strain of C. burnetii in various wild animal tissue. This is an extension of that study with guinea pigs and white mice being investigated. Feces, urine, blood and reproductive tracts (testes or ovaries and uterus), which had not been studied previously were investigated, as were spleen, kidney and liver.

Animals were infected ip with ca. 10^4 guinea pig ID_{50} doses of C. burnetii, then sacrificed at 3, 6, 9 and 12 weeks and the various tissues removed to be tested for presence of rickettsiae.

Table 1. Determination of x-irradiation LD_{50}'s. Data expressed as deaths occurring in guinea pigs, white mice and deer mice within 21 days after whole-body x-irradiation.

Dose Received	Animal Species			LD_{50}*
	Guinea pigs	White mice	Deer mice	
125 r	1/4	-	-	
150 r	1/4	-	-	
175 r	3/4	-	-	163
200 r	3/4	-	-	(Guinea pigs)
225 r	4/4	-	-	
250 r	4/4	-	-	
275 r	4/4	-	-	
300 r	4/4	-	-	
325 r	4/4	0/10	0/10	
350 r	4/4	0/10	0/10	
375 r	4/4	2/10	1/10	
400 r	4/4	2/10	2/10	
425 r	-	2/10	1/10	431
450 r	-	8/10	1/10	(White mice)
475 r	-	10/10	1/10	
500 r	-	10/10	0/10	
525 r	-	10/10	1/10	
550 r	-	10/10	2/10	
575 r	-	10/10	3/10	588
600 r	-	10/10	3/10	(Deer mice)
625 r	-	10/10	9/10	
650 r	-	-	7/10	
675 r	-	-	9/10	
700 r	-	-	10/10	

*LD_{50} calculated using the method of Reed and Muench (1938).

Results are summarized in Table 2. Each animal species will be discussed separately, due to the variation demonstrated between species.

Guinea pigs: Q fever rickettsiae were found at 3 weeks after inoculation in all tissues tested except blood. Urine and fecal material also contained sufficient rickettsiae to incite a high (>1:256) Phase II CF antibody response in sub-inoculated guinea pigs. Organisms could not be demonstrated after the 6 week testing period in spleens, reproductive tracts (mainly female), or livers. Renal tissue still contained C. burnetii at the 12 week period, however, at 9 weeks this pooled tissue incited no detectable antibody response in the sera of the indicator guinea pigs tested.

White mice: Results were similar to those found in guinea pigs concerning blood, kidney, spleen, urine and fecal material. Rickettsiae persisted in livers through 9 weeks and in reproductive tracts (male only) and kidneys through 12 weeks.

Deer mice: Results were virtually identical to those described earlier (Sidwell, 1961), with spleens, kidneys and livers containing Q fever rickettsiae through 6 weeks. No rickettsiae could be demonstrated in urine or blood at any of the times listed.

III. EFFECTS OF WHOLE BODY X-IRRADIATION UPON LATENT COXIELLA BURNETII INFECTION

A. Effect Upon Reactivation of Infection

Guinea pigs, white mice and deer mice were inoculated ip with 0.2 ml of a 10^{-5} dilution of C. burnetii-yolk sac slurry. This was equivalent to ca. 10^{4} guinea pig ID_{50} (Sidwell, 1961). All animals were then held 90 days after which they were divided into three

Table 2. Persistence of rickettsiae in guinea pigs, white mice and deer mice injected intraperitoneally with 10^4 guinea pig ID_{50} Coxiella burnetii.

Animal	Length of Persistence (Weeks)[1]						
	Spleen	Kidney	Reprod. Tr.	Liver	Urine	Feces	Blood
Guinea pigs	6	12[2]	6	6	3	3	3
White mice	6	12[2]	12	9	3	3	3
Deer mice	6	6	3	6	3	3	3

[1] As determined by 28 day Q fever Phase II CF antibody response in tissue-injected indicator guinea pigs.

[2] No rickettsiae at 9 weeks in animals tested, but detected at 12 weeks.

groups: Group I were controls, which were infected but not irradiated; Group 2 were infected animals that received a low dose of x-irradiation; Group 3 were infected animals that received a high dose of x-irradiation.

The time of irradiation was labeled day 0. Subsequent from this time, five animals of each group were sacrificed and bled on day 3, 8, 19, 28 and 49. Spleens, kidneys, reproductive tracts (pooled testes and semen or uterus and ovaries), liver, urine, feces, and blood were removed from each animal and processed for determination of C. burnetii (see Materials and Methods, IV). Serum from each animal was tested for presence of Q fever antibody with the three C. burnetii antigens described previously (see Materials and Methods, III).

Each animal species varied in reactivation results, hence results of studies on each species will be described separately. The results are indicated in Tables 3, 4 and 5 for guinea pigs, white mice and deer mice, respectively. Each of the animals tested were shown in these tables in order to note whether any other tissue in the same animals became infected and to differentiate between sexes.

Guinea pigs: Only one type of organ, the kidney, in control animals continued to contain detectable C. burnetii 90 days after time of infection. This organ was infected in one to three animals at each sacrifice period except the last, at which time all organs and other material were apparently free of demonstrable rickettsiae. Three and 8 days after receiving 100 r x-irradiation, guinea pigs could be shown to contain C. burnetii in their spleen, kidneys, reproductive tract and liver. Nearly all the infected animals were females. On

Table 3. Presence of Coxiella burnetii in guinea pigs receiving low, high or no whole body x-irradiation 90 days after injection

Controls (0 r)

Time[1]	3					8					19					28					49				
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Animal Sex	♀	♀	♀	♂	♂	♀	♀	♀	♀	♂	♀	♀	♀	♂	♂	♀	♀	♂	♂	♂	♀	♀	♀	♂	♂
Spleen	**																								
Kidney				+		÷	÷				+		÷	+			+								
Rep. Tract																									
Liver																									
Urine																	*					*			
Feces									*							*					*				
Blood																									

Low X-Ray (100 r)

Time[1]	3					8					19					28					49				
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Animal Sex	♀	♀	♀	♀	♂	♀	♀	♀	♀	♂	♀	♀	♀	♂	♂	♀	♀	♂	♂	♂	♀	♀	♂	♂	♂
Spleen		÷	+		+		+	+	÷		+	÷			+	÷		+							
Kidney		+	+			+	+	+	+		+				+	+		+	+	+					
Rep. Tract	+	+					+	+	+							+		+							
Liver	+	+	+			+	+	+	+		+				+	+		+							
Urine										*															
Feces			*								+								*				*		
Blood																									

High X-Ray (175 r)

Time[1]	3					8					19					28					49
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Animal Sex	♀	♀	♀	♂	♂	♀	♀	♀	♀	♂	♀	♀	♀	♂	♂	♀	♀	♀	♂	♂	
Spleen						+		+													(All died
Kidney	+		+	+			+	+	+			+	+	+			+		+		prior to
Rep. Tract	+		+		+	+		+	+								+				test)
Liver			+			+		+	+			+	+								
Urine														*							
Feces																	*				
Blood																					

* Indicator guinea pigs died before time of bleeding. Others reinjected, also died.
** Space indicates rickettsiae were not demonstrated in tissue.
[1] Expressed as days after irradiation

Table 4. Presence of Coxiella burnetii in white mice receiving low, high or no whole-body x-irradiation 90 days after infection.

Controls (0 r)

Time[1]	3					8					19					28					49				
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Animal Sex	♀	♂	♂	♂	♂	♀	♀	♂	♂	♂	♀	♂	♂	♂	♂	♀	♀	♀	♂	♂	♀	♀	♀	♂	♂
Spleen	**																								
Kidney																								+	
Rep. Tract								+		+			+	+	+										
Liver																									
Urine			*									*													
Feces	*								*		*	*						*	*				*		
Blood																									

Low X-Ray (300 r)

Time[1]	3					8					19					28					49				
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Animal Sex	♀	♂	♂	♂	♂	♀	♀	♂	♂	♂	♀	♀	♂	♂	♂	♀	♀	♂	♂	♂	♀	♀	♀	♂	♂
Spleen		+						+	+																
Kidney																									
Rep. Tract								+	+				+												
Liver		+						+																	
Urine																					*				
Feces				*				+		*			+	+	*					*					*
Blood																									

High X-Ray (450 r)

Time[1]	3					8					19					28					49				
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Animal Sex	♀	♂	♂	♂	♂	♀	♂	♂	♂	♂	♀	♀	♀	♂	♂	♀	♀	♀	♂	♂	♀	♀	♂	♂	♂
Spleen																									
Kidney																									
Rep. Tract			+				+		+	+															
Liver							+		+																
Urine												*													
Feces							*		+									*					*		
Blood									+																

*Indicator guinea pigs died before time of bleeding. Others reinjected, also died.

**Space indicates rickettsiae were not demonstrated in tissue

[1]Expressed as days after irradiation.

Table 5. Presence of Coxiella burnetii from deer mice receiving low, high or no whole body x-irradiation 90 days after injection.

Controls (0 r)

Time[1]	3					8					19					28					49				
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Animal Sex	♀	♀	♀	♂	♂	♀	♀	♀	♂	♂	♀	♀	♀	♀	♂	♀	♀	♀	♀	♂	♀	♀	♀	♂	♂
Spleen	**																								
Kidney																									
Rep. Tract																									
Liver																									
Urine																									
Feces							*										*								
Blood																									

Low X-ray (350 r)

Time[1]	3					8					19					28					49				
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Animal Sex	♀	♀	♀	♂	♂	♀	♀	♀	♂	♂	♀	♀	♂	♂	♂	♀	♀	♀	♀	♂	♀	♀	♂	♂	♂
Spleen																					+	+			
Kidney																					+	+			
Rep. Tract																									
Liver																							+		
Urine																									
Feces		*				*																			
Blood																									

High X-ray (625 r)

Time[1]	3					8					19					28					49				
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Animal Sex	♀	♀	♀	♀	♂	♀	♀	♀	♀	♂	♀	♀	♀	♀	♂	♀	♀	♀	♂	♂	♀	♀	♀	♂	♂
Spleen																+		+			+			+	
Kidney		+			+											+									
Rep. Tract						+															+				
Liver					+																				
Urine																									
Feces												*													
Blood																+					+				

* Indicator guinea pigs died before time of bleeding. Others reinjected, also died.
** Space indicates rickettsiae were not demonstrated in tissue.
[1] Expressed as days after irradiation.

the 19th day spleen, liver and one sample of feces were infective, both male and female guinea pigs being involved. At the 28th day spleen, kidneys, reproductive tract (male and female), liver and a urine sample contained the Q fever rickettsiae, the positive urine sample coming from a male whose kidneys were also infective. No rickettsiae could be detected in the 100 r irradiated animals by the last sacrifice period. A total of eight guinea pigs died following irradiation by the final sacrifice period.

Those guinea pigs receiving 175 r of x-ray yielded essentially the same results as were obtained with the low dose-irradiated animals, except that fewer tissues were infected at any sacrifice period and no urine or fecal material contained detectable rickettsiae. All animals receiving the high amount of irradiation had died by the 49 day sacrifice period. A total of 28 of the high dose irradiated guinea pigs died by the final sacrifice period.

Guinea pigs receiving either low or high dosages of irradiation generally showed infection with Q fever organism in more tissues than in the control animals, i.e., the indication was that a definite dissemination of C. burnetii to other tissue had occurred. Splenomegaly was observed only occasionally.

White mice: Control white mice infrequently contained demonstrable C. burnetii in male reproductive tissue and in the kidneys. One mouse receiving the low (300 r) x-irradiation treatment had demonstrable rickettsiae in its reproductive tract (male) and spleen at 3 days. By day 8, low dose-irradiated mice contained infective spleen, male reproductive tract, liver and feces. Coxiella burnetii could be

found only in a male reproductive tract and two feces samples on day 19. No organisms could be demonstrated in the low-irradiated group of mice by 28 and 49 days after irradiation.

Tissue infectivity in white mice treated with high x-irradiation doses (450 r) varied little from the non-irradiated control animals, with the exception of two hepatic tissue specimens being infected at 8 days, one of the animals at this time also having a rickettsemia and Q fever organisms in its fecal material. At no time during the study did females have detectable organisms in their tissues and other tested material, although females were examined at all sampling periods. Splenomegaly could not be demonstrated in the three white mouse groups.

Deer mice: No rickettsiae could be demonstrated in control deer mice during the study. Among deer mice receiving low dosages (350 r) of whole body x-irradiation, only two, sacrificed at 49 days after irradiation, exhibited C. burnetii in their viscera. Spleen, kidneys and a liver specimen were infected. Animals treated with 625 r of x-irradiation could sporadically have rickettsiae demonstrated in their organs, i.e., two kidneys and a liver on day 3, a female reproductive tract on day 8, two spleens, a kidney and a blood sample at 28 days and two spleens, a female reproductive tract and a blood sample at 49 days following irradiation.

Spleen enlargement was often noted upon necropsy of irradiated deer mice. Microscopic examination of Machiavello-stained smears of the positive spleens and liverdescribed above revealed visable rickettsiae (Figure 1).

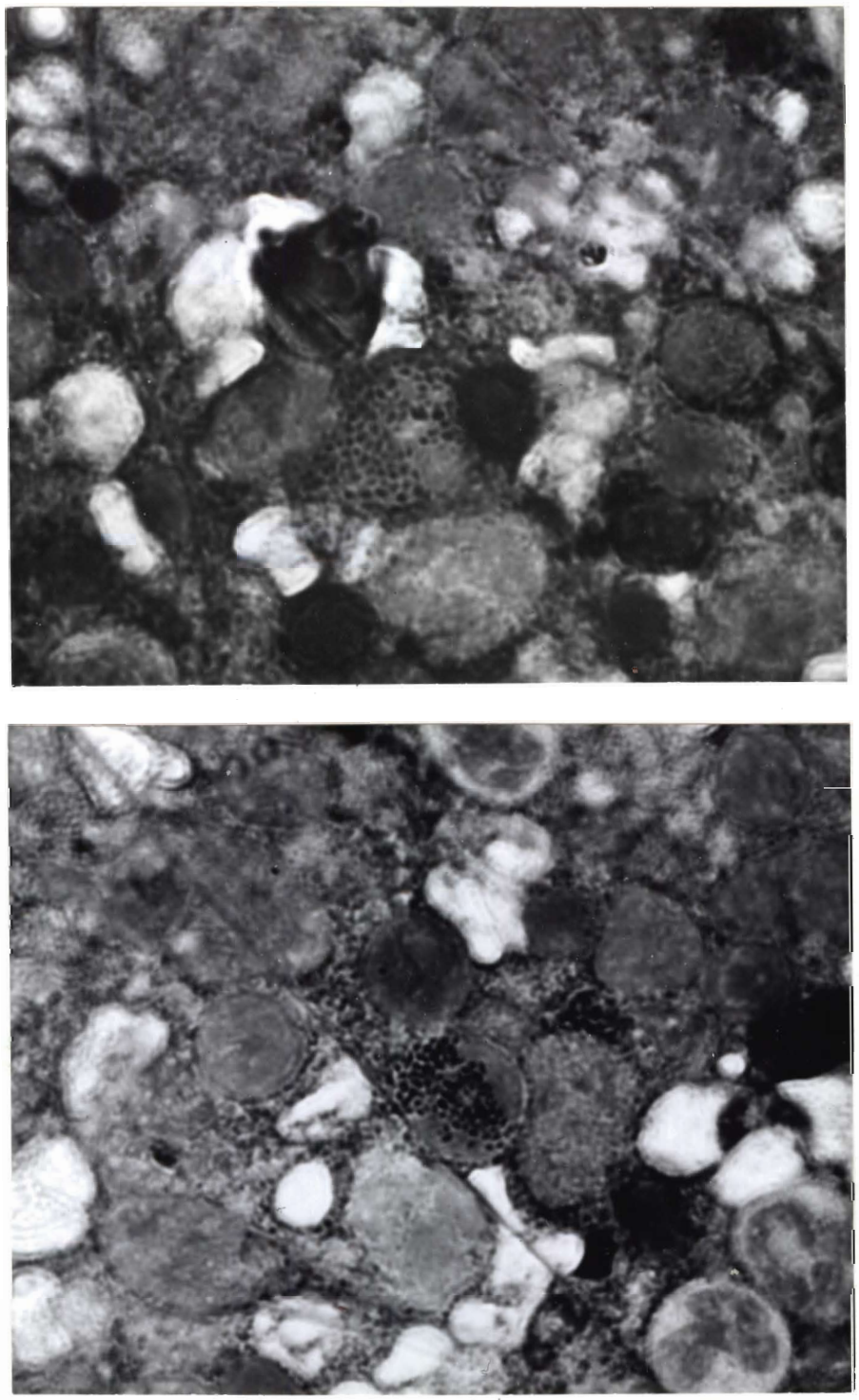

Figure 1. *Coxiella burnetii* in Machiavello-stained tissue smears. Top: liver; bottom: spleen. X 2000.

Indicator guinea pigs injected with urine or fecal material from any of the three animal species frequently died before the time set for bleeding. Others were reinjected with the same material and some of these also died. Further injections were not attempted in the case of deaths occurring a second time, since little of the original material remained for subsequent tests.

B. Effect Upon Q Fever Antibody Response

At each sacrifice period in the reactivation experiment just described, the serum was extracted from the blood of each test animal. Each serum sample was then tested for presence and titer of Phase I and II CF and CTA Q fever antibody. All tests were performed at the same time for closer comparison of titers. The antibody titers of each animal are indicated in Tables 6, 7 and 8. The mean titers of each group are shown graphically in Figures 2, 3 and 4.

Guinea pigs: Little marked variation in titer could be noted in the Phase II CF antibody, except at 28 days after irradiation, at which time the group receiving 100 r of x-ray showed a drop in titer. All guinea pigs in the 175 r x-irradiation group had died prior to the final bleeding, which was 49 days. The same response occurred in Phase I CF antibody; at 28 days, the mean low dose guinea pigs decreased, while the high dose had a grossly increased titer. The same trend was noted with CTA antibody also. At 8 days, the irradiated guinea pigs had higher titers of this antibody, while the converse had occurred by the 49th day. A significant drop in titer occurred among control animals with all three types of antibody between day 28 and 49.

Table 6. Q fever antibody titers[1] in guinea pigs subjected to high, low or no whole-body x-irradiation 90 days after intraperitoneal injection with *Coxiella burnetii*.

Days after Irrad.	Animal No.	Total Irradiation 0 r	100 r	175 r
Phase II Complement Fixing Antibody Titers				
3	1	256	128	32
	2	256	64	512
	3	128	128	256
	4	256	128	64
	5	32	2048	64
8	6	64	64	32
	7	512	256	128
	8	128	64	32
	9	512	1024	512
	10	256	256	128
19	11	512	512	32
	12	512	512	64
	13	64	256	512
	14	256	32	256
	15	256	512	128
28	16	1024	64	1024
	17	1024	64	1024
	18	128	64	2048
	19	1024	128	512
	20	256	32	1024
49	21	64	64	*
	22	64	128	*
	23	64	32	*
	24	32	64	*
	25	64	64	*
Phase I Complement Fixing Antibody Titers				
3	1	128	32	64
	2	128	64	256
	3	64	128	512
	4	32	64	128
	5	64	128	64
8	6	128	64	16
	7	512	512	512
	8	128	32	128
	9	128	1024	512
	10	256	128	128
19	11	256	256	32
	12	256	512	64
	13	64	512	256
	14	128	8	128
	15	128	256	128

(Continued, next page)

Table 6, Continued.

Days after Irrad.	Animal No.	Total Irradiation 0 r	100 r	175 r
Phase I Complement Fixing Antibody Titers (cont'd)				
28	16	128	64	1024
	17	256	64	512
	18	128	64	1024
	19	256	128	512
	20	256	32	512
49	21	64	64	*
	22	128	128	*
	23	64	32	*
	24	32	64	*
	25	64	64	*
Phase I Capillary Tube Agglutination Antibody Titers				
3	1	64	16	8
	2	32	32	64
	3	32	64	128
	4	16	32	32
	5	8	64	32
8	6	32	64	16
	7	32	128	128
	8	16	32	64
	9	16	256	256
	10	32	64	64
19	11	256	256	128
	12	256	256	64
	13	64	128	256
	14	256	8	256
	15	128	128	64
28	16	256	32	256
	17	128	16	256
	18	128	64	512
	19	256	64	128
	20	256	16	256
49	21	64	16	*
	22	64	8	*
	23	32	32	*
	24	32	16	*
	25	32	16	*

[1]Expressed as reciprocals of serum titers.
*Died prior to time of bleeding.

Table 7. Q fever antibody titers[1] in white mice subjected to high, low or no whole-body x-irradiation 90 days after intraperitoneal injection with Coxiella burnetii.

Days after Irrad.	Animal No.	Total Irradiation 0 r	300 r	450 r
Phase II Complement Fixing Antibody Titers				
3	1	64	16	16
	2	32	64	8
	3	8	32	32
	4	64	64	32
	5	64	32	16
8	6	32	32	8
	7	128	16	0[2]
	8	8	32	0
	9	16	8	0
	10	8	16	8
19	11	64	8	64
	12	8	16	16
	13	16	16	32
	14	32	8	32
	15	16	16	32
28	16	32	16	16
	17	32	32	16
	18	32	16	32
	19	32	16	8
	20	32	16	16
49	21	32	32	32
	22	16	32	128
	23	32	64	128
	24	32	32	64
	25	16	8	128
Phase I Complement Fixing Antibody Titers				
3	1	16	8	8
	2	16	8	0
	3	8	8	32
	4	16	16	16
	5	16	8	16
8	6	16	64	0
	7	0[2]	0[2]	0
	8	0	16	0
	9	16	64	0
	10	0	0	0
19	11	8	0	16
	12	8	8	0
	13	8	0	0
	14	8	0	8
	15	8	8	0
28	16	16	16	0
	17	0	8	0
	18	16	0	16
	19	8	0	8
	20	8	8	0

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Table 7, Continued.

Days after Irrad.	Animal No.	Total Irradiation 0 r	300 r	450 r
Phase I Complement Fixing Antibody Titers (cont'd)				
49	21	8	8	16
	22	0	0	8
	23	0	0	8
	24	0	0	32
	25	8	0	32
Phase I Capillary Tube Agglutination Antibody Titers				
3	1	8	0	0
	2	0	8	8
	3	8	0	32
	4	0	8	32
	5	8	8	16
8	6	8	32	8
	7	0	32	16
	8	8	16	8
	9	0	8	16
	10	8	8	8
19	11	16	0	16
	12	0	8	0
	13	8	0	0
	14	0	0	8
	15	8	8	0
28	16	16	16	0
	17	0	16	32
	18	32	0	16
	19	16	0	8
	20	8	8	8
49	21	0	0	0
	22	0	0	0
	23	0	0	8
	24	0	0	0
	25	0	0	8

[1]Expressed as reciprocals of serum titers.
[2]0 indicates a titer of less than 1:8.

Table 8. Q fever antibody titers[1] in deer mice subjected to low, high or no whole-body x-irradiation 90 days after intraperitoneal injection with Coxiella burnetii.

Days after Irrad.	Animal No.	Total Irradiation 0 r	350 r	625 r
Phase II Complement Fixing Antibody Titers				
3	1	32	128	32
	2	64	64	256
	3	16	128	128
	4	64	128	64
	5	128	64	64
8	6	128	32	64
	7	64	64	16
	8	512	32	16
	9	64	128	64
	10	64	128	32
19	11	64	64	64
	12	32	64	64
	13	16	64	128
	14	64	64	64
	15	32	32	64
28	16	32	32	64
	17	32	128	64
	18	128	32	64
	19	64	64	32
	20	64	32	128
49	21	32	32	64
	22	64	64	32
	23	32	64	64
	24	32	64	64
	25	32	32	32
Phase I Complement Fixing Antibody Titers				
3	1	64	64	16
	2	32	64	64
	3	16	32	16
	4	64	64	8
	5	64	32	16
8	6	32	8	32
	7	32	64	16
	8	64	32	8
	9	16	32	16
	10	64	64	8
19	11	16	32	32
	12	8	32	16
	13	16	16	64
	14	32	32	64
	15	64	16	32

(Continued, next page)

Table 8, Continued

Days after Irrad.	Animal No.	Total Irradiation 0 r	350 r	625 r
Phase I Complement Fixing Antibody Titers (cont'd)				
28	16	16	32	32
	17	16	64	32
	18	32	16	16
	19	32	64	16
	20	32	16	32
49	21	16	32	32
	22	32	32	16
	23	16	32	32
	24	16	32	32
	25	16	32	16
Phase I Capillary Tube Agglutination Antibody Titers				
3	1	8	16	8
	2	16	32	16
	3	8	8	32
	4	16	32	8
	5	8	16	16
8	6	32	8	32
	7	64	64	32
	8	128	64	32
	9	64	128	32
	10	128	128	16
19	11	64	16	64
	12	16	128	32
	13	16	64	128
	14	64	64	64
	15	64	16	64
28	16	8	8	16
	17	8	64	64
	18	32	16	16
	19	16	32	32
	20	64	16	32
49	21	8	8	8
	22	8	64	8
	23	8	8	16
	24	8	16	16
	25	8	8	8

[1]Expressed as reciprocals of serum titers.

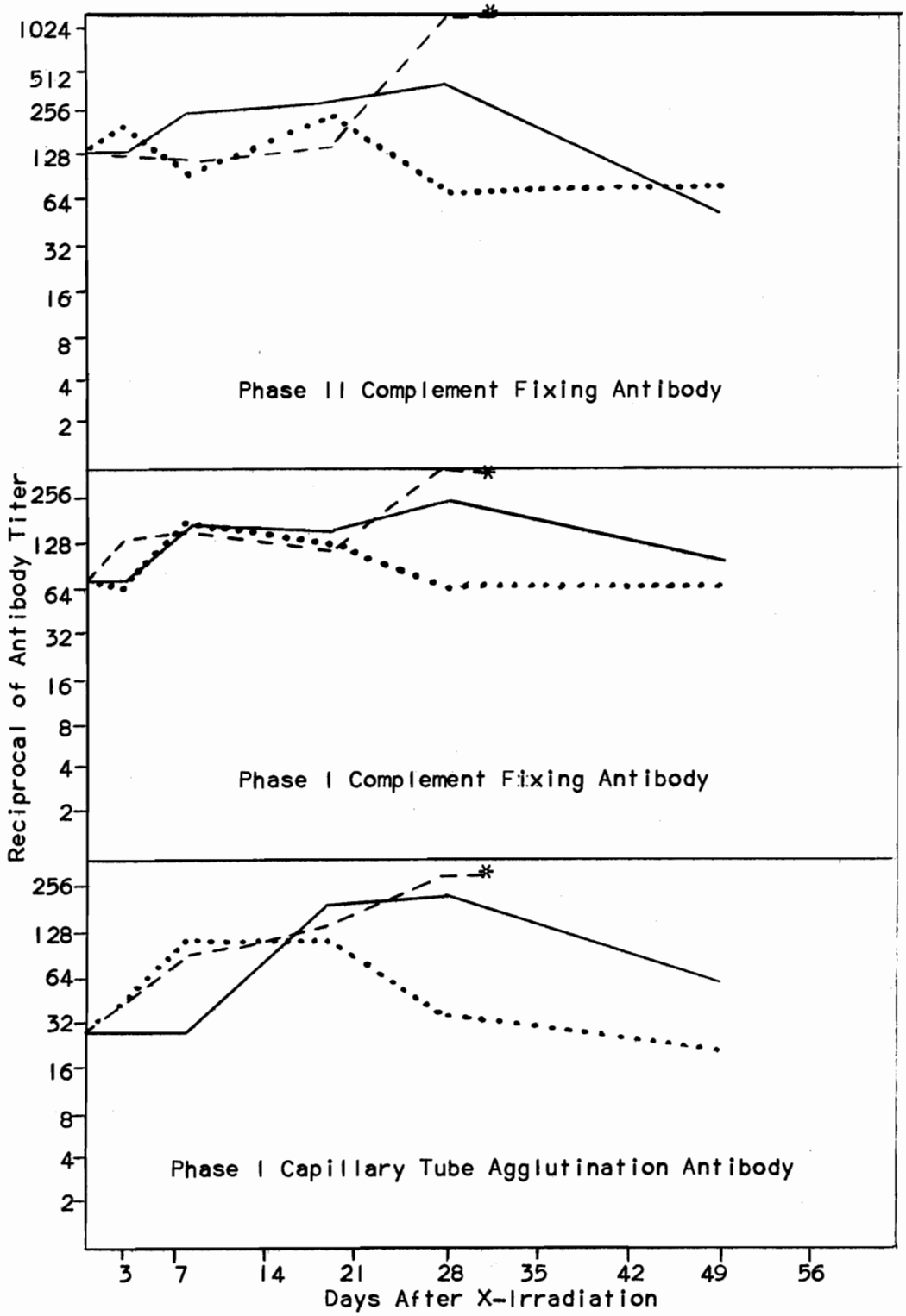

Figure 2. Q fever antibody response in guinea pigs following whole-body x-irradiation 90 days after infection. Data are presented as mean titers at each time of bleeding. Controls (0 r):——; Low X-Ray (100 r):- - -; High X-Ray (175 r):— — —.

* All died prior to time set for final bleeding.

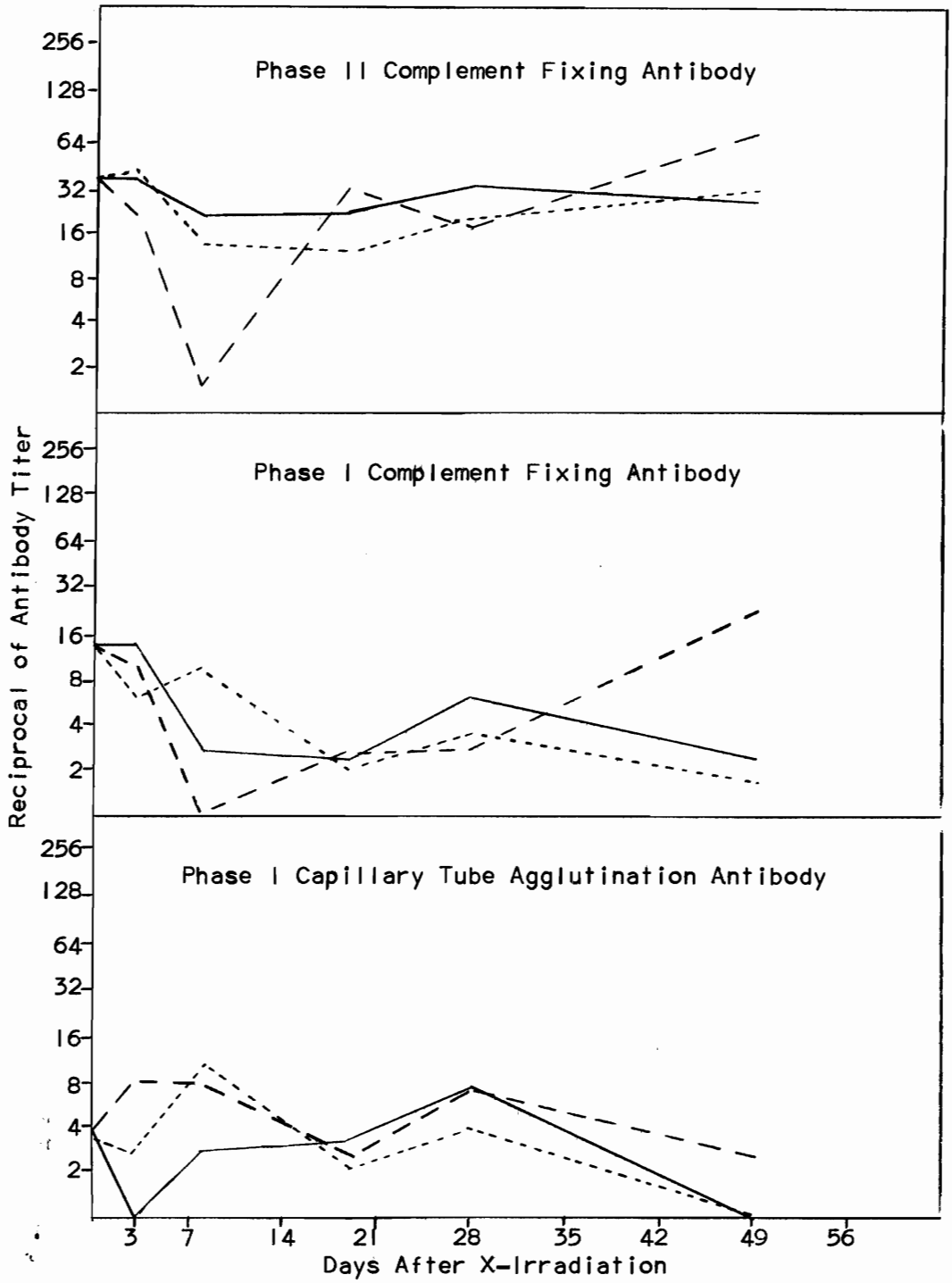

Figure 3. Q fever antibody response in white mice following whole-body x-irradiation 90 days after infection. Data are presented as mean titers at each time of bleeding. Controls (0 r): ———; Low X-Ray (300 r): - - -; High X-Ray (450 r): — — —.

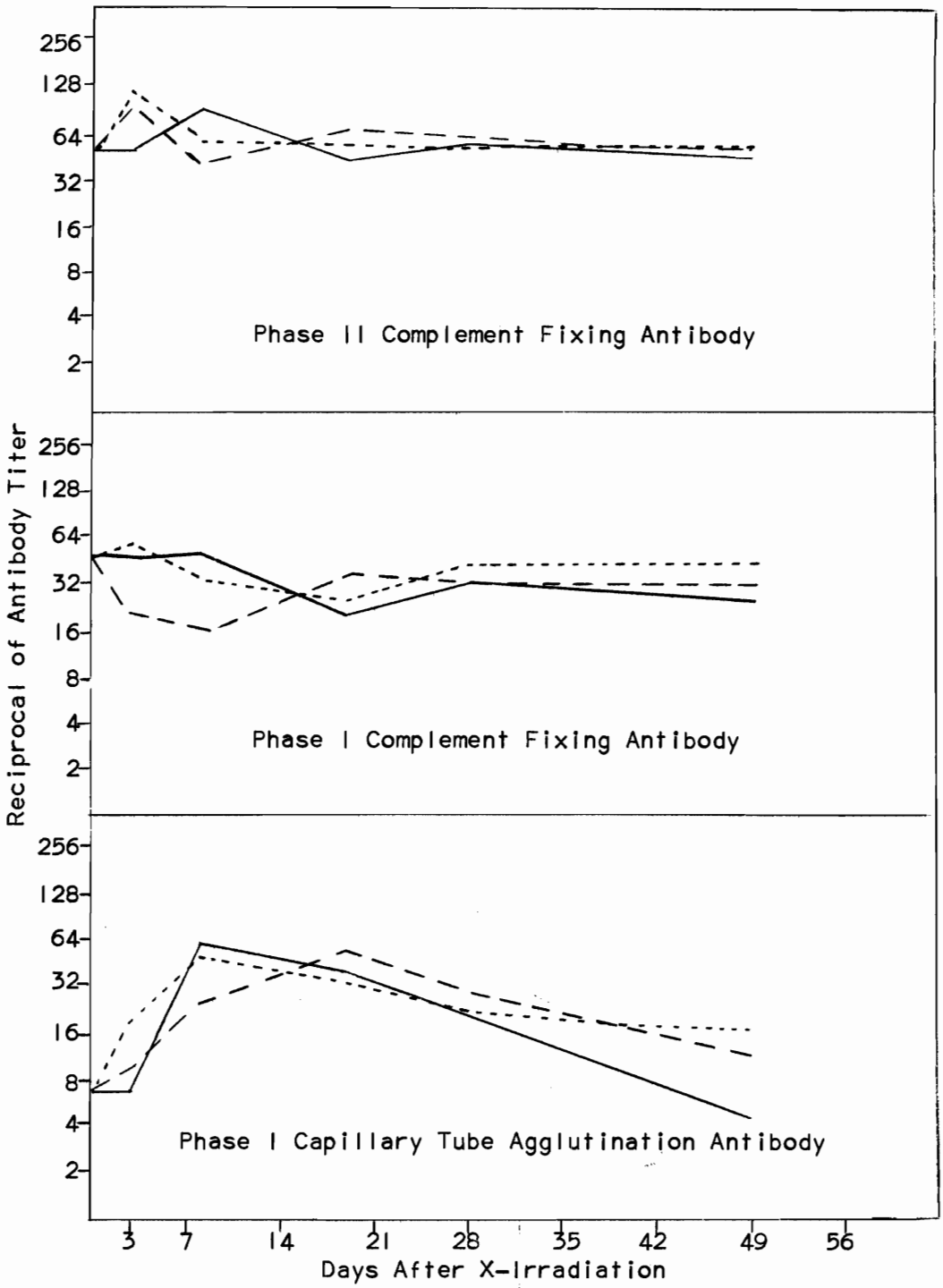

Figure 4. Q fever antibody response in deer mice following whole-body x-irradiation 90 days after infection. Data are presented as mean titers at each time of bleeding. Controls (0 r):———; Low X-Ray (350 r):- - -; High X-Ray (625 r):— — —.

White mice: A marked depression of Phase I and II CF antibody titers occurred in animals receiving high doses of x-irradiation at 8 days. By 19 days, however, the titers in this experimental group had risen and by the 49th day were significantly greater than titers for the other two groups. All Phase I antibody was at a low level (1:8 or less) by the time of irradiation.

Deer mice: Few broad differences could be noted in the groups of deer mouse sera. A trend was noted, however, for all three antibody types. By 8 days after treatment, both irradiated groups had slightly lower mean titers than did the controls. This was reversed, however, by the 49th day, the irradiated animals having higher titers.

The antibody response of the control groups of animals corresponded generally with that for the same period of time reported previously (Sidwell, 1961).

As pointed out earlier, some of the animals, particularly those in the high x-irradiated groups, died before time of sacrifice. When this occurred, the dead animals were dissected and the clotted heart blood was removed and placed in sterile tubes. This was allowed to stand at 4-6°C overnight and then centrifuged at approximately 3,000 rpm for 20 minutes. This separated serum, although extremely hemolyzed, was still found to give good CF and agglutination results and was subsequently used in these studies.

C. Effect Upon the Transmission of Coxiella Burnetii

Six uninoculated control animals of each species were colored and placed in cages with members of each group at time 0. Three animals were of each sex. Eight weeks after this time, all were bled

and their sera tested for Phase I and II CF and CTA Q fever antibody, to determine if any of these control animals had become infected with the disease agent through association with x-irradiated or control infected animals.

Results are shown in Table 9. All control deer mouse sera had no detectable Q fever antibody titers, indicating the animals remained uninfected. Three guinea pigs with the low dose group and four with the high dose group had shown relatively high Phase II CF antibody titers and low Phase I CF and CTA antibody titers indicating that they had become infected during contact with the inoculated animals. Four white mice with the low dose group and two with the high dose group also had similar titers.

IV. EFFECTS OF CORTISONE INJECTIONS UPON LATENT COXIELLA BURNETII INFECTION

A. Effect Upon Reactivation of Infection

Each animal was injected ip with the same concentration of C. burnetii employed in the irradiation study (see Results, III-A). All animals were then held 90 days prior to cortisone treatment. Following this holding period each animal species was divided into three groups: Group I were controls, which were infected but received no cortisone; Group 2 were infected animals which received the low doses of cortisone (1.75 mg total in mice; 14.0 mg total in guinea pigs); Group 3 were infected animals which received the high doses of cortisone (17.5 mg total in mice; 140.0 mg total in guinea pigs). The time of initial cortisone injection was day 0. Following this time, animals were sacrificed and bled on days 3 (3 injections of cortisone were given), 7 (7 injections of

Table 9. Q fever antibody response in normal control animals placed in cages with control and x-irradiated animals injected with Coxiella burnetii 90 days earlier.

Animal	Group	No. Pos. / No. Tested	Titer Range[1]		
			I CF	II CF	CTA
Guinea pig	Control	0/6	0[2]	0[2]	0[2]
	Low x ray	3/6	16-32	128-256	16-32
	High x ray	4/6	16-32	128-512	16-32
White mouse	Control	0/6	0	0	0
	Low x ray	4/6	8-16	64-128	8-16
	High x ray	2/6	8-16	64-128	8-16
Deer mouse	Control	0/6	0	0	0
	Low x ray	0/6	0	0	0
	High x ray	0/6	0	0	0

[1] Reciprocals of antibody titers. I CF=Phase I complement fixing antibody; II CF=Phase II complement fixing antibody; CTA=capillary tube agglutination antibody.

[2] 0 indicates a titer of less than 1:8.

cortisone given), 14, 28, 35 and 63. At each sacrifice period spleen, kidney, reproductive tract (testes or ovaries and uterus), liver, urine, feces and blood were removed from each animal and the presence of C. burnetii determined. Serum from each animal was tested for Q fever antibody using Phase I and II CF and CTA antigens. Results are indicated for individual guinea pigs, white mice and deer mice in Tables 10, 11 and 12, respectively.

Guinea pigs: Control guinea pigs contained infectious quantities of C. burnetii in renal tissue through 153 days, which was the end of the experiment. One spleen at 93 days and a liver at 104 days were also infected. Guinea pigs injected with the low doses of cortisone contained rickettsiae in spleen kidneys, a female reproductive tract, liver and urine. Compared with control guinea pigs, approximately three times as many animals contained infected tissue, indicating a "reactivation", or a spread of the organism from a single organ to other organs. This reactivation occurred primarily 7 to 35 days after initiation of cortisone treatment. The high dosages of cortisone induced reactivation in guinea pigs to a greater extent than the low dosages. Often an animal contained detectable C. burnetii in three and four of the examined organs. Both male and female reproductive tracts were infected in this group. A rickettsemia was demonstrated in a blood sample. Seven guinea pigs injected with cortisone died.

White mice: Control white mice reproductive tracts (male and female) contained demonstrable C. burnetii 90, 97, 104 and 125 days after initial infection. One non-treated mouse had infected kidneys and reproductive tract at 125 days after infection. Injections of the low concentrations of cortisone caused a slight increase in number of

Table 10. Presence of Coxiella burnetii in guinea pigs following injections with low or high dosages of cortisone or in controls, 90 days after infection.

Controls (0 mg cortisone)

Time[1]	3					7					14					28					35					63				
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Animal Sex	♂	♂	♀	♀	♀	♂	♂	♂	♀	♀	♂	♂	♀	♀	♀	♂	♂	♂	♀	♀	♂	♂	♂	♀	♀	♂	♂	♀	♀	♀
Spleen	**	+																												
Kidney	+		+			+							+					+					+			+				
Rep. Tract																														
Liver													+																	
Urine																														
Feces		*										*																		
Blood																														

Low Cortisone (14.0 mg)

Time[1]	3					7					14					28					35					63				
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Animal Sex	♂	♂	♂	♀	♀	♂	♂	♂	♀	♀	♂	♂	♀	♀	♀	♂	♂	♂	♀	♀	♂	♂	♂	♀	♀	♂	♂	♀	♀	♀
Spleen							+		+		+		+			+	+				+		+				+			
Kidney		+		+									+																	
Rep. Tract									+																					
Liver									+				+			+							+							
Urine		+																					+							
Feces								*											*											
Blood																														

High Cortisone (140.0 mg)

Time[1]	3					7					14					28					35					63				
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Animal Sex	♂	♂	♂	♀	♀	♂	♂	♀	♀	♀	♂	♂	o	♀	♀	♂	♂	♀	♀	♀	♂	♂	♀	♀	♀	♂	♂	♂	♀	♀
Spleen	**							+		+			+	+					+				+				+			
Kidney			+					+	+			+	+	+					+				+				+	+		
Rep. Tract									+				+			+												+		
Liver			+						+	+				+					+				+			+	+			
Urine													+										+							
Feces				*													*											*		
Blood										+																				

* Indicator guinea pigs died before time of bleeding. Others reinjected, also died.
** Space indicates rickettsiae were not demonstrated in tissue.
[1] Expressed as days after irradiation.

Table II. Presence of Coxiella burnetii in white mice following injections with low or high dosages of cortisone or in controls, 90 days after infections

Controls (0 mg cortisone)

Time[1]	3					7					14					28					35					63				
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Animal Sex	♂	♂	♀	♀	♀	♂	♀	♀	♀	♀	♂	♀	♀	♀	♀	♂	♂	♀	♀	♀	♂	♂	♀	♀	♀	♂	♂	♀	♀	♀
Spleen	**																													
Kidney																						+								
Rep. Tract						+		+			+											+								
Liver																														
Urine																														
Feces				*														*									*			
Blood																														

Low Cortisone (1.75 mg)

Time[1]	3					7					14					28					35					63				
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Animal Sex	♂	♂	♀	♀	♀	♂	♂	♀	♀	♀	♂	♂	♀	♀	♀	♂	♂	♂	♀	♀	♂	♂	♀	♀	♀	♂	♂	♂	♀	♀
Spleen	+												+				+												+	
Kidney																	+													
Rep. Tract	+						+						+				+													
Liver						+	+																+						+	+
Urine																														
Feces		*						*										*						*						
Blood								+															+							

High Cortisone (17.5 mg)

Time[1]	3					7					14					28					35					63
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
Animal Sex	♂	♂	♀	♀	♀	♂	♂	♂	♀	♀	♂	♂	♀	♀	♀	♂	♂	♂	♀	♀	♂	♂	♂	♀	♀	
Spleen							+	+			+	+						+								
Kidney								+										+						+		(All died
Rep. Tract				+			+					+						+								prior to
Liver	+		+	+		+	+	+			+	+						+						+		test)
Urine	+																									
Feces		*					+					*					*				*					
Blood								+																		

* Indicator guinea pigs died before time of bleeding. Others reinjected, also died.

** Space indicates rickettsiae were not demonstrated in tissue.

[1] Expressed as days after irradiation

Table 12. Presence of Coxiella burnetii from deer mice following injections with low or high doses of cortisone or in controls, 90 days after infection.

Controls (0 mg cortisone)

Time[1]	3					8					14					28					35					63				
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Animal Sex	♂	♂	♀	♀	♀	♂	♂	o	♀	♀	♂	♂	♂	♀	♀	♂	♂	♀	♀	♀	♂	♂	o	♀	♀	♂	♂	♀	♀	♀
Spleen	**																													
Kidney																														
Rep. Tract																														
Liver																														
Urine																														
Feces																		*												
Blood																														

Low Cortisone (1.75 mg)

Time[1]	3					8					14					28					35					63				
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Animal Sex	♂	♂	♂	♀	♀	♂	♂	♀	♀	♀	♂	♂	♂	♀	♀	♂	♂	♂	♀	♀	♂	♂	♀	♀	♀	♂	♂	♂	♀	♀
Spleen																														
Kidney																														
Rep. Tract																														
Liver																														
Urine																														
Feces							*																							
Blood																														

High Cortisone (17.5 mg)

Time[1]	3					8					14					28					35					63				
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Animal Sex	♂	♂	♂	♀	♀	♂	♂	♀	♀	♀	♂	♂	♂	♀	♀	♂	♂	♀	♀	♀	♂	♂	♀	♀	♀	♂	♂	♀	♀	♀
Spleen																														
Kidney																														
Rep. Tract																														
Liver																														
Urine																														
Feces																		*												
Blood																														

* Indicator guinea pigs died before time of bleeding. Others reinjected, also died.

** Space indicates rickettsiae were not demonstrated in tissue.

[1] Expressed as days after irradiation.

infected white mouse organs. Approximately one animal of five at each sacrifice period contained C. burnetii in more than one organ, the principle viscera being male reproductive tract, splenic, hepatic and renal tissue. A rickettsemia was demonstrated on day 7 in a single animal receiving low amounts of cortisone. Injections of high cortisone dosages resulted in more reactivation of infection, one to three white mice in each sacrifice group containing infected tissue. The involved organs were the same as for the low dosage animals, although one urine sample and one fecal material specimen were also infective at day 3 and 7, respectively. A blood sample from a mouse receiving the high dosages of cortisone was infective on day 7 also. Twenty one of 50 white mice died following the high dosage cortisone injections, and by day 63 there were none remaining that could be tested. A total of eight of 50 white mice died in the low dosage group.

Deer mice: Coxiella burnetii could not be demonstrated in deer mouse tissue in any of the three groups of animals and no splenomegaly was observed upon autopsy. No deaths occurred following cortisone injection.

As occurred in the x-irradiation experiment, indicator guinea pigs often died following injection of urine or feces. If reinjection of the same material also resulted in the death of an indicator animal, no further injections were attempted, because of insufficient test material.

B. Effect Upon Q Fever Antibody Response

Serum from each test animal in the reactivation experiment employing cortisone was tested for Phase I and II CF and Phase I CTA Q fever antibody. The antibody titers of each animal are indicated in Tables 13, 14 and 15 for guinea pigs, white mice and deer mice, respectively. Graphical presentations of the mean titers at each time tested are shown in Figures 5, 6 and 7.

Guinea pigs: Among the cortisone-treated animals the serum titers of Phase I and II CF antibody rose significantly compared to control animal sera by the third day after treatment initiation. By day 7 the control sera titers had risen approximately to the same level as those of the treated animals, and no further marked differences were noted except at day 35, when sera from guinea pigs receiving the high cortisone dosages exhibited a drop in Phase I and II CF titers. Only slight titer differences were noted at the experiment termination at 63 days. No gross variations could be seen among the CTA antibody titers, with the exception of the third day, when there was slight drop in titer in sera from cortisone-treated animals.

White mice: Antibody titers in sera from white mice varied erratically between the three groups. The most significant trend noted was a rapid drop in detectable CF titers in the cortisone-treated groups. Little CTA antibody titer variation could be demonstrated. All the high dose cortisone-treated white mice died prior to the 63 day bleeding period.

Deer mice: A decrease in Phase I CF antibody occurred in the sera from deer mice treated with the high doses of cortisone. This

Table 13. Q fever antibody titers[1] in guinea pigs following injections with low or high doses of cortisone or in controls, 90 days after intraperitoneal injection with Coxiella burnetii.

Days after treatment[2]	Animal No.	Total Cortisone per Animal		
		0 mg.	14.0 mg.	140.0 mg
Phase II Complement Fixing Antibody Titers				
3	1	32	128	1024
	2	64	128	1024
	3	32	128	64
	4	64	128	256
	5	32	64	512
7	6	512	64	512
	7	512	512	128
	8	128	128	128
	9	64	128	256
	10	64	64	512
14	11	128	32	128
	12	64	128	64
	13	64	128	64
	14	64	128	128
	15	128	64	128
28	16	64	32	32
	17	128	64	32
	18	64	64	64
	19	32	128	128
	20	64	64	64
35	21	16	32	128
	22	128	32	16
	23	32	256	0[3]
	24	64	256	0
	25	64	64	64
63	26	128	256	128
	27	32	256	16
	28	128	16	64
	29	128	64	128
	30	128	32	32
Phase I Complement Fixing Antibody Titers				
3	1	32	256	64
	2	64	128	256
	3	0[3]	128	128
	4	64	128	256
	5	16	32	128
7	6	128	32	256
	7	256	256	64
	8	32	128	16
	9	64	128	256
	10	32	128	128
14	11	64	64	64
	12	32	64	32
	13	32	64	32
	14	0	64	64
	15	16	32	64

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Table 13, Continued

Days After Treatment[2]	Animal No.	Total Cortisone per Animal		
		0 mg	14.0 mg	140.0 mg
Phase I Complement Fixing Antibody Titers (cont'd).				
28	16	64	64	32
	17	32	64	16
	18	32	32	32
	19	8	128	8
	20	16	64	16
35	21	128	32	32
	22	64	32	16
	23	8	32	0
	24	32	128	0
	25	16	64	32
63	26	256	128	128
	27	16	128	32
	28	32	0	32
	29	128	32	64
	30	64	16	64
Phase I Capillary Tube Agglutination Antibody Titers				
3	1	16	16	0
	2	16	32	0
	3	16	0	32
	4	16	16	16
	5	32	0	8
7	6	32	0	32
	7	64	64	16
	8	16	16	16
	9	16	32	32
	10	16	16	16
14	11	16	16	16
	12	16	32	16
	13	16	32	32
	14	0	16	16
	15	32	16	32
28	16	32	32	16
	17	16	16	32
	18	16	64	64
	19	32	64	32
	20	16	32	32
35	21	32	32	64
	22	64	64	32
	23	64	64	32
	24	32	64	64
	25	16	16	32
63	26	64	64	64
	27	32	64	16
	28	16	8	64
	29	64	8	64
	30	32	16	16

[1]Expressed as reciprocals of serum titers
[2]Beginning with the initial injection of cortisone
[3]0 indicates a serum titer of less than 1:8

Table 14. Q fever antibody titers[1] in white mice following injections with low or high doses of cortisone or in controls, 90 days after intraperitoneal injection with Coxiella burnetii.

Days after treatment[2]	Animal No.	Total Cortisone per Animal		
		0 mg	1.75 mg	17.5 mg
Phase II Complement Fixing Antibody Titers				
3	1	16	64	0[3]
	2	16	32	0
	3	16	64	0
	4	64	0[3]	0
	5	32	16	0
7	6	8	32	0
	7	8	16	*
	8	0[3]	0	0
	9	16	0	0
	10	8	8	0
14	11	8	0	32
	12	8	0	*
	13	16	0	16
	14	16	*	0
	15	16	0	*
28	16	16	0	*
	17	16	16	*
	18	8	16	16
	19	32	8	0
	20	32	0	0
35	21	0	0	0
	22	0	0	0
	23	0	0	8
	24	16	*	0
	25	32	*	*
63	26	8	0	*
	27	32	*	*
	28	16	*	*
	29	128	0	*
	30	16	0	*
Phase I Complement Fixing Antibody Titers				
3	1	0	64	0
	2	32	64	0
	3	0	32	0
	4	32	0	0
	5	16	16	0
7	6	0	64	0
	7	0	16	*
	8	8	0	0
	9	32	0	0
	10	16	8	0
14	11	0	0	32
	12	0	0	*
	13	0	16	16
	14	16	*	0
	15	8	0	*

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Table 14, Continued

Days After Treatment[2]	Animal No.	Total Cortisone per Animal		
		0 mg	1.75 mg	17.5 mg
Phase I Complement Fixing Antibody Titers (cont'd)				
28	16	0	0	*
	17	0	0	*
	18	8	8	8
	19	16	8	0
	20	16	0	0
35	21	0	0	0
	22	0	0	8
	23	0	0	16
	24	32	*	0
	25	16	*	*
63	26	16	0	*
	27	8	*	*
	28	16	*	*
	29	16	0	*
	30	8	0	*
Phase I Capillary Tube Agglutination Antibody Titers				
3	1	0	16	0
	2	0	0	0
	3	0	16	0
	4	16	0	0
	5	0	8	0
7	6	0	0	0
	7	0	0	*
	8	8	0	0
	9	0	0	0
	10	0	0	0
14	11	0	0	0
	12	8	0	*
	13	0	0	16
	14	16	*	0
	15	0	0	*
28	16	0	0	*
	17	0	8	*
	18	0	16	8
	19	8	16	8
	20	16	0	0
35	21	16	32	0
	22	16	16	0
	23	32	32	8
	24	16	*	0
	25	8	*	*
63	26	8	16	*
	27	8	*	*
	28	16	*	*
	29	8	32	*
	30	16	16	*

[1]Expressed as reciprocals of serum titers
[2]Beginning with the initial injection of cortisone
[3]0 indicates a serum titer of less than 1:8
*Died prior to time of bleeding

Table 15. Q fever antibody titers[1] in deer mice following injections with low or high doses of cortisone or in controls 90 days after intraperitoneal injection with *Coxiella burnetii*.

Days after Treatment[2]	Animal No.	Total Cortisone per Animal		
		0 mg	1.75 mg	17.5 mg
Phase II Complement Fixing Antibody Titers				
3	1	128	128	64
	2	256	64	64
	3	128	64	128
	4	32	64	64
	5	64	64	64
7	6	32	128	16
	7	64	32	16
	8	64	64	64
	9	64	128	64
	10	64	128	32
14	11	64	128	64
	12	64	64	32
	13	32	64	16
	14	64	64	64
	15	32	64	32
28	16	64	128	64
	17	32	64	32
	18	32	64	32
	19	64	128	16
	20	32	64	32
35	21	16	256	16
	22	32	64	64
	23	64	128	64
	24	32	64	16
	25	32	128	32
63	26	16	128	32
	27	32	16	16
	28	32	16	8
	29	8	32	32
	30	16	64	16
Phase I Complement Fixing Antibody Titers				
3	1	64	32	32
	2	64	32	64
	3	64	64	128
	4	32	64	32
	5	64	32	64
7	6	32	128	16
	7	64	16	8
	8	64	64	16
	9	64	64	32
	10	64	64	16
14	11	64	128	16
	12	32	64	32
	13	16	64	16
	14	32	32	32
	15	32	64	32
28	16	64	128	32
	17	32	64	32

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Table 15, Continued

Days after Treatment[2]	Animal No.	Total Cortisone per Animal		
		0 mg	1.75 mg	17.5 mg
Phase I Complement Fixing Antibody Titers (cont'd)				
28	18	16	64	16
	19	32	64	8
	20	32	64	16
35	21	32	256	0[3]
	22	32	64	0
	23	32	256	64
	24	16	0[3]	0
	25	64	32	8
63	26	32	64	0
	27	16	16	0
	28	16	16	0
	29	16	32	16
	30	16	16	8
Phase I Capillary Tube Agglutination Antibody Titers				
3	1	16	0	0
	2	32	0	16
	3	16	16	16
	4	16	16	0
	5	16	0	0
7	6	0[3]	16	0
	7	16	0	0
	8	16	0	0
	9	16	0	16
	10	8	8	8
14	11	0	16	0
	12	0	0	0
	13	0	0	0
	14	8	0	0
	15	8	8	8
28	16	0	8	8
	17	16	16	0
	18	0	16	8
	19	0	0	0
	20	16	8	8
35	21	16	128	16
	22	32	32	16
	23	64	64	32
	24	16	16	16
	25	32	32	8
63	26	16	32	8
	27	8	16	8
	28	8	8	0
	29	16	16	16
	30	8	16	8

[1]Expressed as reciprocals of serum titers.
[2]Beginning with the initial injection of cortisone.
[3]0 indicates a serum titer of less than 1:8.

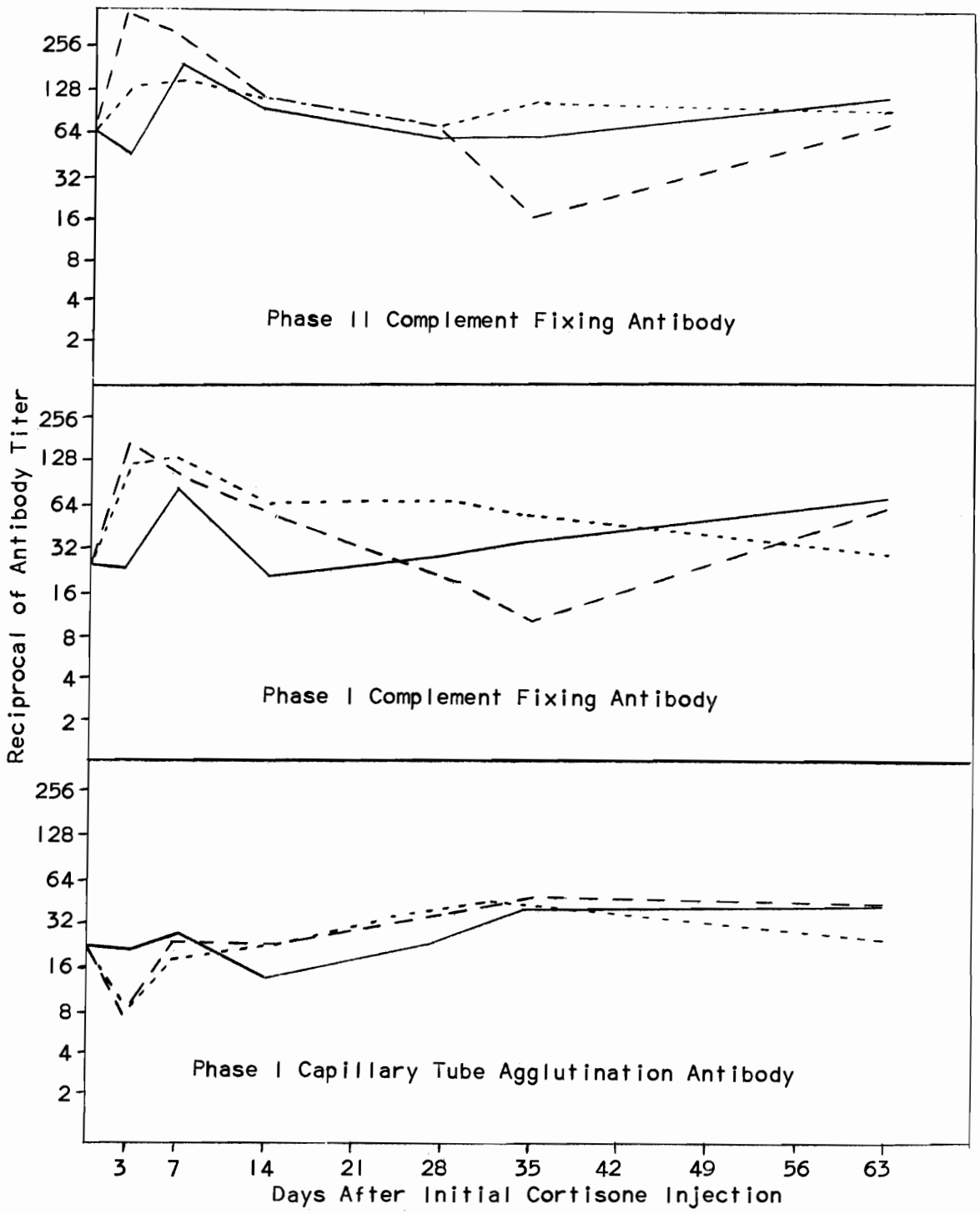

Figure 5. Q fever antibody response in guinea pigs following cortisone injection 90 days after initial infection. Data are presented as mean titers at each time of bleeding.
Controls (0 mg cortisone):———; Low Cortisone (14.0 mg):- - -; High Cortisone (140.0 mg):— — —

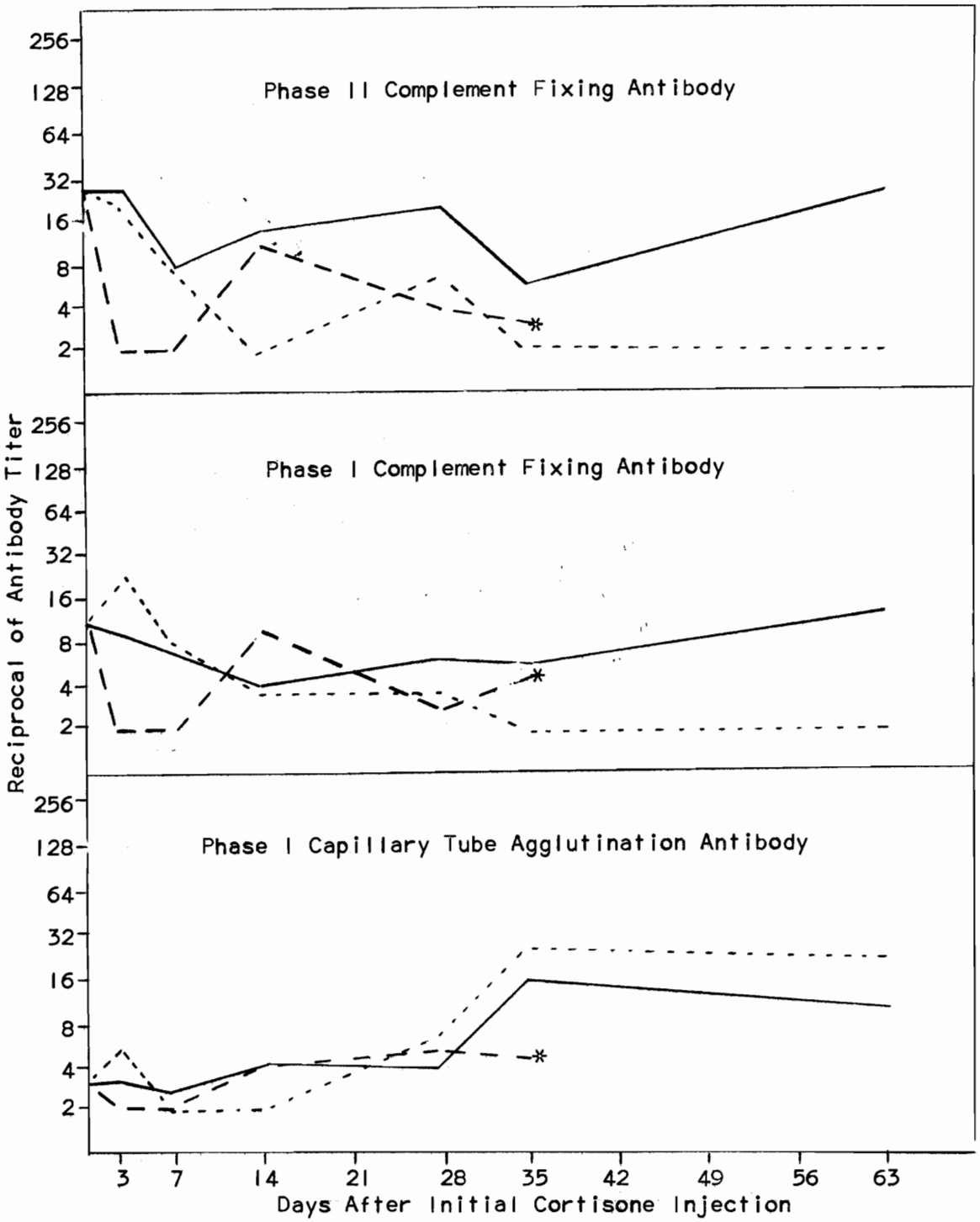

Figure 6. Q fever antibody response in white mice following cortisone injection 90 days after initial infection. Data are presented as mean titers at each time of bleeding.
Controls (0 mg. cortisone):———; Low Cortisone (1.75 mg):- - - -;
High Cortisone (17.5 mg):— — —.

*All died prior to time set for final bleeding.

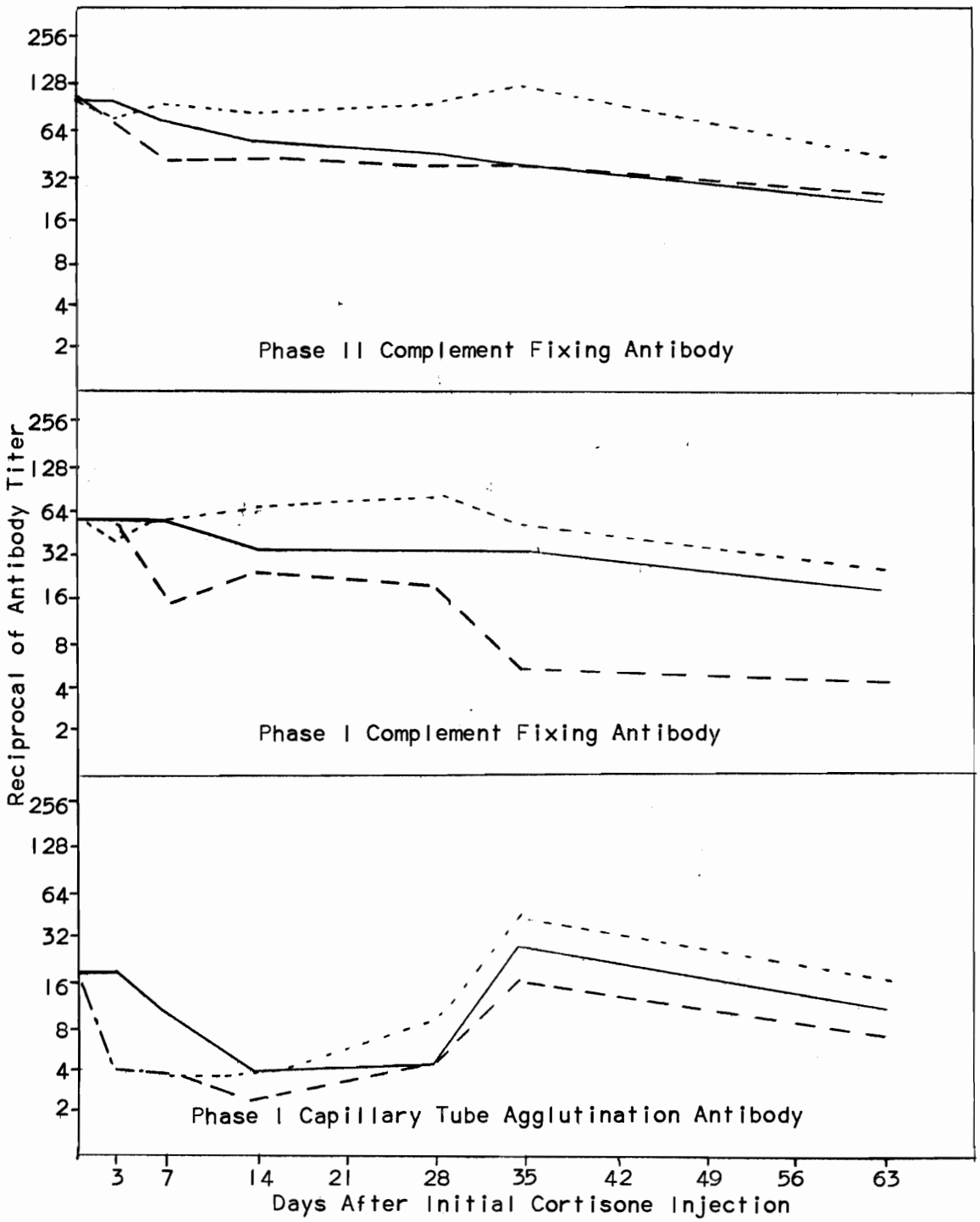

Figure 7. Q fever antibody response in deer mice following cortisone injection 90 days after initial infection. Data are presented as mean titers at each time of bleeding.
Controls (0 mg. cortisone):———; Low Cortisone (1.75 mg):- - - -;
High Cortisone (17.5 mg):— — —.

decrease was seen by day 7, and was maintained the length of the experiment. No other meaningful variations occurred.

C. Effect Upon the Transmission of Coxiella burnetii

Transmission of C. burnetii was demonstrated in a manner analogous to that described in the irradiation experiments (Results, III - C). Results summarized in Table 16 show the titer range of the uninfected control animals placed with the cortisone-treated and non-treated infected animals. Two of six guinea pigs became infected through association with the low dosage cortisone group, and five of six became infected through association with the high dosage group. Among white mice, four of six controls became infected through association with the high dosage cortisone injected group. None became infected with low dosage group. All uninfected animals placed with non-treated infected animals remained uninfected, as determined by antibody response. All deer mice likewise remained uninfected.

V. EFFECT OF PARTURITION UPON LATENT COXIELLA BURNETII INFECTION

White mice and guinea pigs were injected ip with the same concentration of C. burnetii used for x-irradiation and cortisone studies (Materials and Methods, V-C). Forty-five days after infection, the sexes, previously kept separate, were allowed to intermingle. Animals becoming pregnant were isolated and held until birth of young. The offspring borne from the infected parents were sacrificed at varying times up to 20 days after birth. The maternal parents were sacrificed at the sacrifice time of the oldest progeny. Lung, liver, kidney, spleen, reproductive tract, blood, urine and feces were removed when possible from all sacrificed animals, and presence of

Table 16. Q fever antibody response in normal control animals placed in cages with control and cortisone injected animals infected with Coxiella burnetii 90 days earlier.

Animal	Group	No. Pos. / No. Tested	Titer Range[1]		
			I CF	II CF	CTA
Guinea pig	Control	0/6	0[2]	0[2]	0[2]
	Low cort.	2/6	8-32	128-512	8-64
	High cort.	5/6	16-32	128-512	16-64
White mouse	Control	0/6	0	0	0
	Low cort.	0/6	0	0	0
	High cort.	4/6	16-32	128-256	8-32
Deer mouse	Control	0/6	0	0	0
	Low cort.	0/6	0	0	0
	High cort.	0/6	0	0	0

[1] Reciprocals of antibody titers. I CF=Phase I complement fixing antibody; II CF=Phase II complement fixing antibody; CTA=Phase I capillary tube agglutination antibody.

[2] 0 indicates a titer of less than 1:8.

C. burnetii determined by guinea pig injection. Individual tissues from the parent animals were tested since a spread of the rickettsiae to other organs was to be studied. Offspring tissue was pooled for each individual animal. Sera from all animals were tested for Q fever antibody using the Phase I and II CF and Phase I CTA antigens.

Guinea pigs: Results are presented in Table 17. Ten guinea pigs became pregnant during the experiment and four of these gave birth to a total of seven partus containing infectious quantitites of C. burnetii in their tissue. In every maternal parent of these rickettsiae-containing offspring a spread of the Q fever organisms to several organs occurred. The viscera involved in this spread included spleen, kidneys, liver, lung, reproductive tract and mammary glands. Urine and feces also contained C. burnetii in one of the parents, and a rickettsemia was demonstrated in two of the parent animals. Individual tissues were not investigated in the young guinea pigs, since the tissue and other material, was pooled for each animal. In five of the seven positive progeny the tissue pools induced Q fever Phase II CF antibody titers of greater than 1:256 in the sera of the indicator guinea pigs. Antibody titers increased one to two tube dilutions in all the formerly pregnant animals, as compared to infected non-pregnant controls during the same periods of time. Nearly every partus displayed relatively high antibody titers to C. burnetii, but always in lower levels than observed in the parents. The antibody titers varied inversely with the age of the young animals.

Table 17. Effect of parturition upon reactivation of *Coxiella burnetii* infection in guinea pigs.

Maternal Parent						Offspring				
Animal No.	Days after infection	Infected tissue[1]	Antibody Titer[2] II CF	I CF	CTA	Age (days)	Antibody Titer[2] II CF	I CF	CTA	Tissue infectivity[3]
	89					8	128	8	16	0[4]
1	89	K	512	128	128	8	256	16	16	0
	93					3	128	64	32	0
	93					3	64	32	16	0
2	110	-	512	128	32	14	64	16	8	0
	105	S K L Lu				1	128	32	8	256
3	110	R F U M B	512	256	128	5	64	8	8	256
4	120	-	256	256	32	1	128	64	8	0
	125					2	64	8	8	0
5	131	K	512	128	32	8	32	8	8	0
	127					1	256	128	32	64
6	134	S L	1024	1024	256	8	64	8	8	256
7	130	S K L R M B	256	64	32	1	64	16	8	64
	130					2	64	32	32	0
8	138	K R	256	128	128	10	32	8	8	0
	134					1	256	64	16	0
9	149	-	512	128	32	15	32	16	16	0
	138					1	32	8	8	256
10	149	S K L R	128	64	16	21	16	8	8	256

[1] S=spleen; L=liver, Lu=lung; R=reproductive tract; K=kidney; F=feces; U=urine; B=blood; M=mammary glands; -=no infected tissue detected.

[2] Reciprocals of Q fever antibody titers. II CF=Phase II complement fixing antibody; I CF=Phase I complement fixing antibody; CTA=Phase I capillary tube agglutination antibody.

[3] Reciprocals of antibody titers induced by offspring tissue injected into indicator guinea pigs.

[4] 0 indicates a titer of less than 1:8.

White mice: Results are indicated in Table 18. Of eight parent mice investigated, two gave birth to progeny which may have had infected tissues. Parent number 1 had a litter of five mice. These were sacrificed at ages from 1 day to 20 days. Of the five, only one, which was sacrificed when 20 days old, induced Q fever antibody in an indicator guinea pig. This induced antibody was comparatively low (1:32). Parent number 5 gave birth to a litter of seven mice, which were sacrificed at ages 1 day to 12 days. Two 1-day old mice and one of three 5-day old mice were considered to have infectious tissue, inciting titers of 1:16, 1:32 and 1:8 respectively in the indicator guinea pigs. Both parents of C. burnetii-infected partus contained the rickettsiae in several organs. These organs were spleen, kidneys, liver and reproductive tract. One other parent contained the Q fever organism in the reproductive tract. One other parent contained the Q fever organism in the reproductive tract and in feces, while a fourth previously pregnant white mouse had an infected liver. No significant change occurred in Q fever antibody titer in the sera of any parent mice, and no progeny contained demonstrable antibody titers in their sera.

VI. SECONDARY ANTIBODY RESPONSE

To attempt to clarify the meaning of the Q fever antibody titers demonstrated in the reactivation experiments, 16 guinea pigs were injected ip with the same amount of C. burnetii used earlier for the reactivation experiments. Ninety days after infection these animals were bled from the orbital sinus and their sera tested for antibody titers to the three antigens employed. After bleeding, each

Table 18. Effect of parturition upon reactivation of Coxiella burnetii infection in white mice.

Maternal Parent						Offspring				
Animal No.	Days after infection	Infected tissue[1]	Antibody Titer[2] II CF	Antibody Titer[2] I CF	Antibody Titer[2] CTA	(days)	Antibody Titer[2] II CF	Antibody Titer[2] I CF	Antibody Titer[2] CTA	Tissue infectivity[3]
	81					1	0[4]	0[4]	0[4]	0[4]
	81					1	0	0	0	0
	85					5	0	0	0	0
	89					8	0	0	0	0
1	110	S L R K	64	0[4]	0	20	0	0	0	0
	89					2	0	0	0	0
	89					2	0	0	0	0
	89					2	0	0	0	0
	103					15	0	0	0	0
	103					15	0	0	0	0
2	103	L	128	0	0	15	0	0	0	0
	103					1	0	0	0	0
	110					7	0	0	0	0
3	110	R F	0	0	0	7	0	0	0	0
	110					2	0	0	0	0
	110					2	0	0	0	0
	119					11	0	0	0	0
	128					20	0	0	0	0
4	128	-	0	0	0	20	0	0	0	0
	119					1	0	0	0	16
	119					1	0	0	0	32
	123					5	0	0	0	0
	123					5	0	0	0	0
	123					5	0	0	0	0
	130					12	0	0	0	0
5	130	S K L	128	16	0	12	0	0	0	0

(Continued, next page)

Table 18, Continued

Maternal Parent						Offspring				
Animal No.	Days since infection	Infected tissue[1]	Antibody Titer[2] II CF	I CF	CTA	Age (days)	Antibody Titer[2] II CF	I CF	CTA	Tissue infectivity[3]
	123					1	0[4]	0	0	0[4]
	123					1	0	0	0	0
	130					8	0	0	0	0
	130					8	0	0	0	0
6	130	-	0[4]	0	0	8	0	0	0	0
	130					1	0	0	0	0
	130					1	0	0	0	0
7	130	-	32	16	8	1	0	0	0	0
	132					1	0	0	0	0
8	136	-	16	0	0	5	0	0	0	0

[1] S=spleen; L=liver; R=reproductive tract; K=kidney; F=feces; -=no infected tissue detected

[2] Reciprocals of Q fever antibody titers. II CF=Phase II complement fixing antibody; I CF=Phase I complement fixing antibody; CTA=Phase I capillary tube agglutination antibody.

[3] Reciprocals of antibody titers induced by offspring tissue injected into indicator guinea pigs.

[4] 0 indicates a titer of less than 8.

animal was reinjected ip with the same quantity of C. burnetii used earlier. Four of the guinea pigs were inoculated ip, four sc in the inguinal region and four intravenously (iv) by cardiac puncture. Four were not reinfected, and served as controls. Each animal was then bled at day 3, 7, 14, 21, 28, 35 and 56 after time of reinjection, the method of bleeding being again from the orbital sinus. The Q fever antibody titers in each serum sample were then determined.

The individual Q fever antibody titers of each guinea pig serum sample are listed in Tables 19, 20 and 21 for animals injected ip, sc and iv respectively 90 days after original infection. Table 22 indicates titers of control animals which were not reinfected. The mean titers are graphically summarized in Figure 8.

Following the second injection of C. burnetii, the mean titers of the sera from many animals exhibited a slight decrease (ca. one tube dilution) in Q fever antibody titer to all three antigens employed. This was succeeded by a raise of one to two tube dilutions above the original by 7 to 14 days following the second injection. The titers gradually dropped to, or slightly below, the level of the original titers, at times varying according to route of injection. No titer variations occurred greater than two tube dilutions from the non-secondarily infected controls.

One of the iv injected guinea pigs died at 7 days, and two of the remaining three died by 56 days, so the antibody response results are limited in regards to this route of secondary injection.

Table 19. Q fever antibody titers[1] in guinea pigs injected intraperitoneally with *Coxiella burnetii* 90 days after initial infection.

Days after 2nd inj.	Animal Number			
	1	2	3	4
Phase II Complement Fixing Antibody Titers				
0	64	64	128	16
3	64	64	128	16
7	32	64	128	16
14	64	64	256	32
21	128	64	256	16
28	64	32	256	16
35	64	16	256	16
56	64	16	128	*
Phase I Complement Fixing Antibody Titers				
0	32	16	64	16
3	32	16	128	16
7	16	16	32	16
14	16	16	64	16
21	128	32	128	16
28	32	32	64	16
35	32	16	128	16
56	32	16	32	*
Phase I Capillary Tube Agglutination Antibody Titers				
0	16	16	64	16
3	32	16	32	8
7	16	16	16	8
14	16	16	32	8
21	32	16	32	16
28	32	16	32	16
35	32	8	32	8
56	32	16	16	*

[1] Reciprocals of serum titers.
* Died prior to time of bleeding.

Table 20. Q fever antibody titers[1] in guinea pigs injected subcutaneously with *Coxiella burnetii* 90 days after initial infection.

Days after 2nd inj.	Animal Number			
	5	6	7	8
Phase II Complement Fixing Antibody Titers				
0	128	128	128	32
3	64	32	256	64
7	128	64	256	128
14	128	64	256	128
21	64	64	128	64
28	32	32	128	64
35	32	16	128	*
56	32	32	32	
Phase I Complement Fixing Antibody Titers				
0	64	32	32	16
3	32	16	128	16
7	32	16	128	16
14	32	32	128	16
21	32	32	32	16
28	16	16	32	16
35	16	16	64	*
56	16	16	16	
Phase I Capillary Tube Agglutination Antibody Titers				
0	16	32	32	16
3	16	16	32	16
7	32	16	32	16
14	32	16	32	32
21	32	32	32	16
28	16	16	32	16
35	8	32	32	*
56	16	16	16	

[1] Reciprocals of serum titers.
* Died prior to time of bleeding.

Table 21. Q fever antibody titers[1] in guinea pigs injected intravenously with *Coxiella burnetii* 90 days after initial infection.

Days after 2nd inj.	Animal Number			
	9	10	11	12
Phase II Complement Fixing Antibody Titers				
0	256	16	256	128
3	128	32	256	128
7	128	16	*	64
14	256	64		128
21	256	64		128
28	128	32		64
35	*	16		128
56		*		128
Phase I Complement Fixing Antibody Titers				
0	64	16	16	32
3	128	16	16	64
7	64	16	*	16
14	128	16		64
21	128	16		64
28	128	16		64
35	*	16		64
56		*		64
Phase I Capillary Tube Agglutination Antibody Titers				
0	64	16	16	32
3	128	16	16	64
7	64	16	*	16
14	128	16		64
21	128	16		64
28	128	16		64
35	*	16		64
56		*		64

[1] Reciprocals of serum titers.
* Died prior to time of bleeding.

Table 22. Q fever antibody titers[1] in guinea pigs receiving no second injection of Coxiella burnetii (controls).

Days after 2nd inj. of test animals[2]	Animal Number			
	13	14	15	16
Phase II Complement Fixing Antibody Titers				
0	64	64	128	32
3	64	64	128	32
7	64	64	128	64
14	64	128	128	64
21	64	128	128	32
28	64	128	64	32
35	64	64	64	32
56	64	64	64	32
Phase I Complement Fixing Antibody Titers				
0	16	32	32	16
3	16	32	32	16
7	16	32	32	16
14	16	16	32	16
21	16	16	32	16
28	16	32	32	16
35	16	32	32	16
56	16	16	32	16
Phase I Capillary Tube Agglutination Antibody Titers				
0	16	16	32	16
3	16	16	32	16
7	16	32	32	16
14	16	16	32	16
21	16	16	32	16
28	16	32	32	16
35	32	32	32	16
56	32	32	32	16

[1] Reciprocals of serum titers.
[2] See tables 19 to 21.

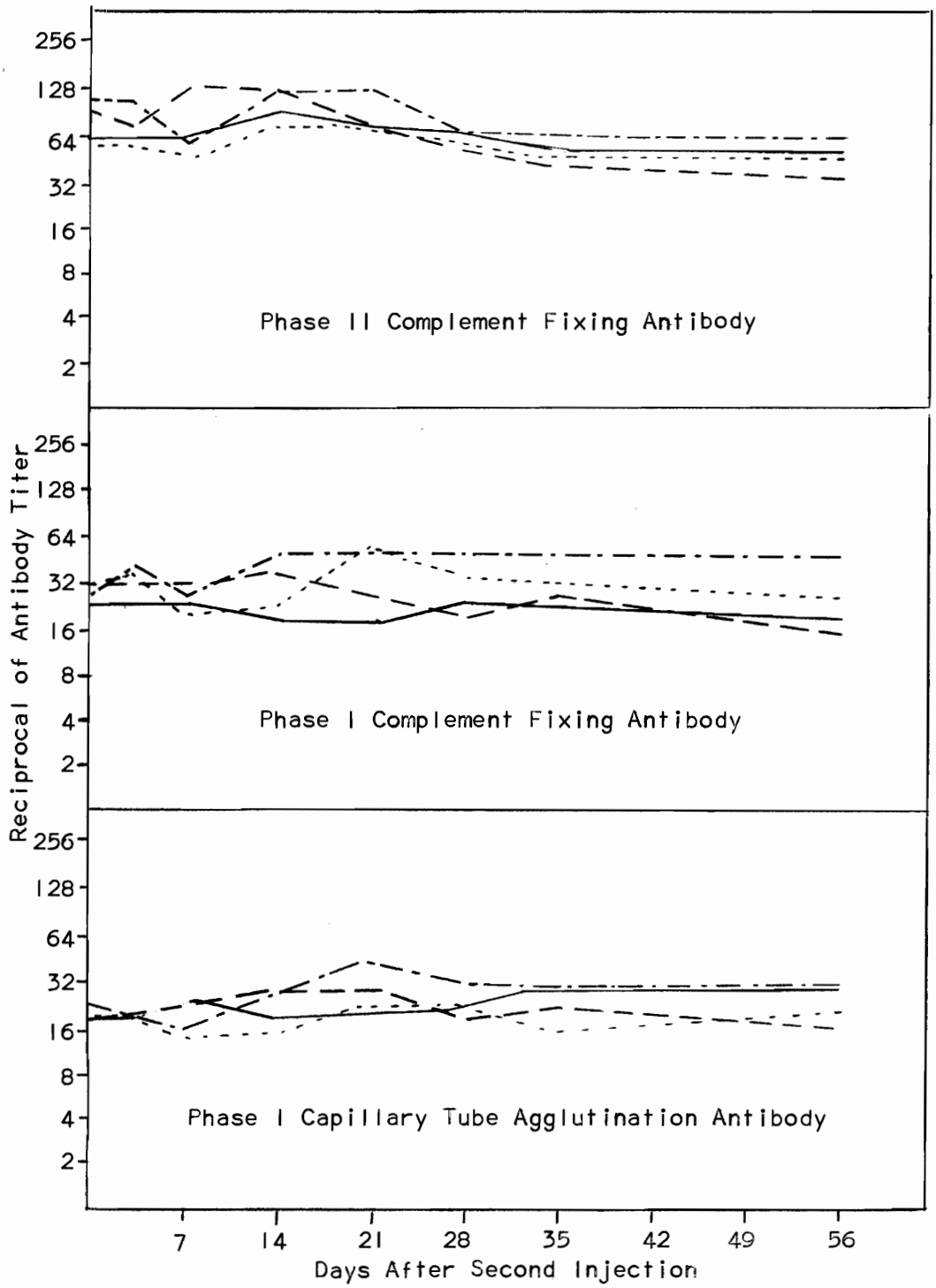

Figure 8. Q fever antibody response in guinea pigs following a second injection of Coxiella burnetii 90 days after initial infection. Data are presented as mean titers at each time of bleeding. Controls (no 2nd inj.)——; Subcutaneous Inj.— — —; Intraperitoneal Inj.· · · ·; Intravenous Inj.—· — ·.

DISCUSSION

The overall primary purpose of these studies was to attempt to reactivate a latent C. burnetii infection in laboratory and wild animals. Three different agents were selected to study their ability to cause a reactivation. These were whole body x-irradiation, multiple cortisone injections and the physiological process of parturition. Since these agents vary in their actions, each will be discussed separately.

X-irradiation: Because the radiation field to be used was somewhat erratic, a pile of lead rings covered by a metal screen was employed, which resulted in a very constant field of irradiation on the surface between the rings. The use of such a radiation surface, however, presumably slows or "softens" the x-rays, so they are not so penetrating, and thus may do more cellular damage.

The LD_{50} results for the laboratory animals obtained in this investigation compare well with data procured by other workers (Donaldson, 1954; Miya, 1959), insofar as this earlier data can be interpreted. According to the available literature, no work has been reported concerning x-irradiation sensitivity of deer mice.

To attempt to reactivate latent Q fever infections two x-irradiation doses were used. A "low" dose was in the range in which no animals, or at least very few would die. The "high" dose was in the range in which over half of the irradiated animals would die. It was apparent from these studies that both x-irradiation dosages were capable of either reviving a latent infection that was not detectable in the viscera, or were capable of causing the infection to spread to other organs of the body. The reactivation was especially apparent in guinea pigs, followed to a lesser extent in white mice and then in deer mice.

These three animal species were susceptible to whole body x-irradiation in the same order, thus the reactivation occurred in direct proportion to the animals' apparent susceptibility to irradiation.

All organs and excrements examined were capable of containing detectable amounts of C. burnetii, the primary animal exhibiting this being the guinea pig. The persistence of the rickettsiae coincides with other reports, Parker and Steinhaus (1943) demonstrating it 40 to 110 days after defervescence in guinea pigs, Reczko (1950) showing persistence in guinea pigs through 526 days, and DeMattia et al. (1952) describing persistence in white mouse kidneys up to 80 days. In the present study, the 3 and 8 day sacrifice periods were chosen to attempt to demonstrate a rickettsemia; the assumption being that, if any reactivation did occur and was initiated rapidly after irradiation, the organism would be carried by the bloodstream to other parts of the body. A rickettsemia was not detected, however, except in one white mouse at 8 days and deer mice at 28 and 42 days. Even so, the blood stream could still be the principal disseminating route of the organism, since the times examined do not represent all times during which a rickettsemia may exist. The lymphatic system could also be involved, but was not studied in this investigation.

In guinea pigs and deer mice the revival of infection was not confined to a single sex, although females appeared to respond earlier than males. Reactivation in white mice, however, was confined solely to males.

Urine and fecal material was shown to contain C. burnetii during reactivation and represents a method by which the agent could be transmitted to other animals. A spread of the rickettsiae was defin-

itely shown when uninfected animals placed as cage mates with infected and irradiated guinea pigs and white mice exhibited Q fever antibody titers in their sera in 8 weeks. The antibody response of these cage controls (high Phase II CF titers, low Phase I CF and CTA titers) indicated that an infection had occurred approximately 3 to 6 weeks earlier, or between the 2nd and 5th weeks after x-irradiation of inoculated animals. This time was based on previous data concerning antibody response (Sidwell, 1961).

Whole body x-irradiation was employed in these studies because of the known effects that such penetrating radiation causes in animals. Death of animals by lethal doses of irradiation are generally thought to be caused by severe diarrhea, accompanied by breakdown, ulceration and necrosis of the intestinal wall. This is followed by excessive sodium loss and injury of surrounding cells by toxins and proteolytic enzymes formed or released subsequent to irradiation. Death has also been attributed to organisms of the intestinal tract which may be released into the body cavities as a result of irradiation. Other effects of such penetrating radiation includes temporary atrophy of liver cells, necrosis of stomach cells, injury to blood vessels (slight but possibly enough to cause general hemorrhaging), cellular degeneration of the spleen, effects to bone marrow (hyperplasia, haematopoiesis, erythroblast destruction, granulocyte decrease), destruction of some lymphatic tissue, edema and hemorrhages in the lungs, hemorrhaging and impairment of tubular functions in the kidneys, and other, more subtle, effects (Hollaender, 1955; Pottinger, 1961). Nearly all of these factors could have an effect upon the reactivation

of a latent rickettsial infection, by altering the infected tissues which would allow the organism to be released to reinfect other parts of the body.

A study of the antibody response would indicate any suppression of antibody-forming mechanisms. The fact, however, that the treated animals had been infected for three months created the problem of whether the observed antibody was continually being produced, or merely persisted in the sera for that period of time. If the latter were the case, then little or no suppression of antibody should be noted. Most cells of the body will be regenerated within 3-5 weeks after irradiation (Pottinger, 1961). If C. burnetii is reactivated, the organism may after a few weeks re-stimulate the antibody-forming mechanisms, resulting in increase in titer.

A week after irradiation of the guinea pig with the low x-ray dose, there was a slight depression of Q fever CF antibody, followed by a significant increase in titer by 28 days. This indicated a depression of antibody-forming cells, followed by regeneration of these cells and a re-stimulation by reactivated C. burnetii. No increase in titer was noted in sera from animals receiving low doses of x-ray, indicating either a suppression of antibody production or not enough organisms reactivated to cause a meaningful re-stimulation, or both. The same general trend was also noted in white mice and deer mice, but to a lesser degree in the latter animals. A factor responsible for a great deal of the erraticness observed in the results was the sacrifice of the animals at each time of testing. Thus the titers indicated represent different animals each time, and fluctuate with the indivi-

dual animals. Only "trends" can therefore be determined from each result. A further discussion of the secondary antibody response will be presented later.

Cortisone treatment: The results obtained in these studies indicated that a series of cortisone injections at the concentrations employed, given as described in this study, can bring about a reactivation of C. burnetii infection in guinea pigs and white mice, but not in deer mice. Reactivation was more apparent in the treated guinea pigs than in the treated white mice. This could not have been due to the differences in concentration of cortisone administrated to each species, since the guinea pigs received less drug per gram of animal weight than did white mice. The dosages used were based on previous work by others. For example, Austin (1957) reported reactivation of psittacosis infection in white mice using 5 mg cortisone daily for 2 days; this approximately equaled the high dose employed in the present study for white mice and deer mice. Nullification of the interference phenomenon in guinea pigs was obtained by Mika et al. (1959) by injecting 10 mg cortisone acetate daily for 7 days, which was intermediate between the low and high dosages employed for the guinea pigs in the present study. Most of the other investigations discussed in the literature review (Sec. V) indicate this same general dosage range.

As was observed in the x-irradiation experiments, each organ and other material examined appeared capable of containing detectable amounts of C. burnetii. The persistence of the organism in the control animals in the cortisone experiments also correlated with its presence in the x-ray study control animals. The blood stream was not definitely

shown to be the means of systemic spreading the rickettsiae, although treated white mice and guinea pigs, in individual instances, did have a rickettsemia at 7 days after initiation of the cortisone injections. The lymphatic system may be implicated, but was not studied in these experiments.

Reactivation of infection occurred in both sexes of guinea pigs and white mice. This differed from results obtained from the x-ray experiments, in which reactivation occurred only in male white mice.

Urine was shown to be infective with C. burnetii in guinea pigs and white mice following cortisone treatment. Only one specimen of feces contained rickettsiae. The sample was from a white mouse. These excretions may have been responsible for the spread of the infection to normal animals placed in the cages with the treated animals. Both sexes of the normal cage mate control animals were infected, as indicated by Q fever antibody response. In no instances did all the normal animals become infected, indicating there was not an extensive aerosol or other contaminating influence prevailing.

Cortisone, or 17-hydroxy-11-dehydrocorticosterone, is an adrenocortical steroid hormone known to be biologically very active. The compound is effectively absorbed following intramuscular or subcutaneous injection, and is especially effective for therapeutic and experimental use if the hormone concentration in the tissues can be maintained relatively constant. This maintenance of concentration is readily accomplished by daily injection because the compound is absorbed at a slow, even rate over a 24 hour period (Goodman and Gilman,

1955). The hormone has a wide variety of actions in the animal body, particularly if present in high concentrations as when administered parenterally. Hypercorticism results in increased carbohydrate metabolism, increased protein metabolism, negative potassium balance, hyperexcitabily, alteration of the response of the primitive mesenchyne to injury or disease, increased androgenic activities and increased resistance to stress (Goodman and Gilman, 1955). All these effects are probably secondary with respect to reactivation of an infection with an organism such as C. burnetii, except as applied to a general tissue breakdown which could release the agent previously confined to a few cells. Excessive cortisone effects such as the above could result in death of the animal. Of primary importance to a reactivation of infection wrought by excess cortisone are effects such as dissolution of lymphocytes in the thymus, lymph nodes, spleen and Peyer's patches (Dougherty and White, 1947), depression of many of the formed elements of the blood other than lymphocytes, e.g. eosinophils, neutrophils and erythrocytes (White and Dougherty, 1945; Thorn et al., 1953), alteration in antibody response (Rose, 1959) and alteration of inflammatory reaction (Dougherty, 1953). There are contradictory reports regarding adrenocortical steroids upon pre-formed antibodies and antibody production. Some investigators have claimed a rise in titer accompaning lymphoid tissue dissolution (Dougherty et al., 1944), while others have reported no differences in titer (Eisen et al., 1947; Fischel, 1950). A depression of antibody formation was demonstrated by Germuth et al. (1951), while Mirick (1951) could not show any decrease in antibody production. Many other conflicting results

have been reported on this same topic (Rose, 1959). It is probable that the conflicting data reported are due to differences in disease studied, time of testing of serum, quantity of hormone administered, time of administration and animal species. The inflammatory response is inhibited by corticosteroids (Dougherty, 1953; Brandon, 1962). This is brought about by a combination of many factors, such as the reduction of vascular permeability, inhibition of profibrinolysin and hyaluronidase action, phagocytic activity depression, inhibition of pyrogen release and many other factors (Brandon, 1962). The combination of tissue alteration, releasing the organism, and depressed immune response would be sufficient to reactivate a formerly latent infection.

The Q fever antibody response was investigated in the present study to show the effects of cortisone upon serum titers in treated animals compared to control animal titers. It was observed that the titers varied according to animal species. Hormone treated guinea pigs exhibited a marked increase in CF antibody titer which was followed by a drop in titer several weeks later. This would indicate a lymphocyte dissolution, releasing excess antibody to the blood, which gradually decreased naturally until sub-normal due to depression of antibody forming mechanisms. Sera from cortisone-injected white mice and deer mice demonstrated a rapid drop in CF antibody titer, which might indicate an active antibody-producing system which was suppressed due to the hormone without a marked dissolution of lymphatics. Results were erratic, as may be expected from individual animals sacrificed at each test period. Capillary tube agglutination antibody varied little in all animals, but this type of test was relatively insensitive.

There is a small basal requirement for cortical hormones in quiet life, the hormones playing a general supporting role rather than an initiating role in bodily processes (Sayers, 1950). Superimposed on this small basal requirement are the needs entailed by various stresses. With a major stress the demand may become extremely large. This then indicates a natural application of the studies of cortisone effects upon latent infections, for every type of stress, if of sufficient magnitude and duration, can affect the adrenal cortex.

Examples of such stresses are exercise (Hatai, 1915), exposure to heat (Ewert, 1934-1935), cold (Hartman et al., 1931), intense light (McKinley and Rivera, 1934), reducedatmospheric pressure (Pincus and Hoagland, 1943), anoxia (Armstrong and Heim, 1938), trauma (Venning et al., 1944), and infection (Hartman and Brownell, 1949). There is also considerable evidence that the adrenals are modified during pregnancy (Guieysse, 1899; Watrin, 1919; Nahm and McKinzie, 1937). Christian (1956) has reported the stress of increased population size causes an enlargement in size and increase in excretions of the adrenal glands. Thus one may postulate that any type of stress which an animal latently infected with C. burnetii may be exposed to could conceivably initiate a reactivation of the infection. This in turn could lead to a renewed outbreak of the disease in a susceptible population.

Parturition: The data presented indicate that pregnancy and/or the act of parturition can cause a reactivation of latent Q fever infection in laboratory animals. This reactivation was especially apparent in guinea pigs, but was only slightly manifest in white mice. The progeny from latently infected parents were also often infected with

C. burnetii. The infection of the offspring may have taken place in any one of several ways. The fetus could have received an infecting dose of C. burnetii transovarally or transplacentally; a rickettsemia was observed in two of the "infection reactivated" parents. A contamination of the surroundings leading to an infection in the new-borne partus may have occurred by means of infective birth fluids; reproductive tracts in four of the guinea pigs and two white mouse parents were observed to contain the Q fever organism. A final method by which the offspring may have received the pathogen could also be attributed to contaminated surroundings as a result of excretions of infective urine and feces.

A reactivation of Q fever infection as a result of parturition is not a new concept. As discussed earlier (Literature Review I-B), Q fever was first reported to occur as a latent infection when it was reactivated during parturition in certain livestock. The data reported in this investigation is apparently the first reported observation of reactivation due to pregnancy or parturition in laboratory animals.

Deer mice were not investigated as a part of the present study because of the difficulty in breeding this type of mammal, particularly following the stress of an experimental infection of C. burnetii. Personal observations prior to the current investigations, however, have shown that occasional offspring borne to infected deer mice parents have never contained detectable Q fever rickettsiae. Similar results have also been observed in the unborn fetus from infected female deer mice.

One may speculate on the mechanisms involved in the infection reactivation process in parturient laboratory animals, especially in view of the results of the cortisone studies discussed earlier. Since pregnancy and parturition are moderated by a hormonal system in the body, and can directly affect the secretions of many hormones, reactivation of infection is undoubtedly brought about by the effects of such hormones, in addition to the stress applied to the reproductive tract tissue. If the tissues of the reproductive tract are altered or damaged to the extent that C. burnetii harbored therein can escape, the various hormones may then act to depress the factors involved in the body's immune response, enabling the organisms to become carried to, and established in, other organs.

A slight stimulation of Q fever antibody response occurred in the guinea pig maternal parents, but no such marked variations could be detected in the sera of infected white mouse parents. All guinea pig offspring, regardless of whether they were infected with demonstrable C. burnetii, contained significant titers of the three Q fever antibodies tested. This would indicate a transfer of the maternal antibody, particularly when antibody titers dropped in the older progeny. No maternal antibody was detectable in offspring of white mice, possibly because only low quantities of antibody existed in the maternal parent. Q fever rickettsiae present in the partus tissue of both guinea pigs and white mice apparently did not incite significant antibody titers, since no differences could be seen between serum titers of infected and seemingly uninfected offspring.

Secondary antibody response: It was speculated that if the antibody titers observed in sera from irradiated or hormone-treated animals were caused partially by the release of rickettsiae into the circulatory systems of the animals, the same type of response might be produced by a secondary injection of C. burnetii. Consequently guinea pigs, which had shown the greatest variation in antibody titer following stress, were re-infected following a 90 day-holding period. Intraperitoneal, sc and iv injections were employed in different groups of animals to determine if the injection route would cause variations in the antibody response.

Following an ip injection of C. burnetii the primary Q fever antibody response in the three animal species tested usually begins within a few days after injection. The titer rapidly increases to a peak in 3 to 7 weeks, depending upon animal and phase of antibody. After reaching this peak, the titers gradually decrease over a period of approximately a year until antibody can no longer be detected (Sidwell, 1961).

When the animals are injected a second time, regardless of route, an antibody titer decrease of ca. one tube dilution generally occurred within 7 days. This was probably due to partial neutralization of the antibody by the additional antigen present in the blood. Following this slight decrease, there was an increase in titer of one to two tube dilutions above the original titer. This increased level of antibody either persisted through the end of the experiment or dropped to the original titer or lower by 4 weeks. This increase typifies the usual secondary reaction as described for animals injected with antigens such as diptheria antitoxin (Glenny, 1925a, 1925b, 1931)

and bacteria such as Salmonella paratyphi (Wilson and Miles, 1955). Employing the latter antigens, however, one obtains much higher titers than those observed in the present investigation. Burnet and Freeman (1938) could induce no secondary rise of antibody in rabbits primarily and secondarily stimulated by iv injection of Q fever rickettsiae. The rabbit is a much less susceptible animal than the guinea pig to Q fever, which may be the reason for the observed antibody fluctuations, although slight, in the guinea pig sera.

The demonstrated secondary antibody response correlated with the guinea pigs which had been subjected to stress in that neither the stressed animals nor the secondarily injected animals demonstrated extreme rises in titer. An exception was the high-dose irradiated guinea pigs, whose serum antibody did rise up to four tube dilutions from the original. Close comparisons cannot be drawn, however, since the secondarily infected animals had not been subjected to any extreme stresses, which probably alters the antibody forming mechanisms of the animals.

Treatment of feces: Those indicator guinea pigs dying after injection of fecal material or urine may have become infected with a penicillin resistant organism from the material. Penicillin can probably cause the death of guinea pigs through reduction of the intestinal flora, leaving a resistant organism which excretes toxins, killing the animal (Cormea et al., 1947; Ambrus et al., 1952; Stevens and Gray, 1953). For this reason the penicillin employed in this experiment was mixed and incubated with the material to be tested prior to injection into the indicator animals. This causes adsorption

of the penicillin before introduction into the animal and reduces the possibility of fatality due directly or indirectly to the antibiotic.

SUMMARY

1. A 21-day whole body x-irradiation LD_{50} was determined for guinea pigs, white mice and deer mice. As measured by post-exposure deaths, guinea pigs were most susceptible (LD_{50}: 163 r), white mice less susceptible (LD_{50}: 431 r) and deer mice least susceptible (LD_{50}: 588 r) to the irradiation.

2. In a pilot rickettsiae persistence study, Coxiella burnetii was shown to persist for over 12 weeks in kidneys of guinea pigs and reproductive tract and kidneys of white mice. Fecal material and urine in guinea pigs, white mice and deer mice were infectious for three weeks. The organism persisted a total of six weeks in deer mouse spleen, kidneys and liver.

3. Whole body x-irradiation in dosages slightly less than or greater than the 21-day LD_{50} caused a reactivation of C. burnetii infection in guinea pigs, white mice and deer mice infected three months previously. This reactivation was determined by demonstrating infectious quantities of rickettsiae in various tissues and in the urine and feces in these animals, as compared to the detection of little or no rickettsiae in the same tissues from non-irradiated similarly infected control animals.

4. The Q fever antibody response in the irradiated and control animals in (3) were shown to vary markedly in titer with animal species, dosage of irradiation and type of antibody. The Phase I and II CF antibody in sera from irradiated guinea pigs was depressed at one week after irradiation, but increased significantly by four weeks in the animals receiving the high irradiation dose. A negative antibody phase occurred in the animals receiving the low dose within four weeks. Phase

l CTA antibody, by contrast, increased in titer by one week, but was depressed subsequently only in the low dosage irradiated animals. White mice reacted to a lesser extent than guinea pigs, but the same general trends in antibody depression and enhancement were noted. Few variations of significance could be demonstrated in sera from deer mice, although one to two weeks after irradiation a slight depression of CF antibody occurred in the irradiated animals.

5. A definite transmission of C. burnetii to control guinea pigs and white mice in relation to reactivation of the Q fever infections in irradiated animals was demonstrated. This was shown by the infection of normal control animals placed as cage mates with the infected and irradiated animals. No transmission could be observed in deer mice and non-irradiated control animals.

6. An injection of cortisone given daily for seven days induced a reactivation of Q fever infection in guinea pigs and white mice but not in deer mice infected three months previously. This reactivation was indicated by the presence of C. burnetii in various tissue, urine and feces which were usually uninfected in untreated animals infected at the same time.

7. The multiple cortisone injections described in (6) had only a moderate effect upon the Q fever antibody response in the three animal species tested. A slight depression in titer occurred in most animals at varying time intervals following the cortisone injections. This was usually preceeded by a sudden increase in CF titer during the treatments.

8. Normal control animals placed as cage mates with cortisone-treated guinea pigs and white mice became infected with C. burnetii.

This indicated that a reactivation of Q fever infection by cortisone treatment can result in a spread of the disease agent. No transmission was observed in deer mice at the cortisone concentrations employed, and all normal cage mates of untreated control animals remained uninfected.

9. A reactivation of Q fever infection occasionally occurred following parturition in guinea pigs and white mice previously infected with C. burnetii. Various viscera and frequently blood, urine and feces from these animals were shown to contain C. burnetii as early as 8 days and as long as 20 days after birth of offspring. Partus, primarily guinea pigs, from these infection-reactivated parents often were also infected. Little change in Q fever antibody titer was noted in parents or progeny.

10. A secondary Q fever antibody response was studied in guinea pigs reinfected with C. burnetii three months after a primary infection. Fluctuations in titer were slight, although many animals exhibited a slight decrease in serum antibody titer to all three antigens employed. This was succeeded by a moderate increase of titer above the original amount by one to two weeks. The titers gradually dropped to, or slightly below, the original titer. Intraperitoneal, subcutaneous and intravenous routes of secondary injection were employed in separate experiments, with little difference noted in induced antibody titers.

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EXPERIMENTAL STUDIES OF LATENT Q FEVER INFECTIONS

by

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Whole body x-irradiation in dosages of slightly less than or greater than the 21 day LD_{50} brought about reactivation of a latent Coxiella burnetii infection in guinea pigs, white mice and deer mice (Peromyscus maniculatus sonoriensis LeConte) infected three months previously. The reactivation was determined by demonstrating infectious quantities of the rickettsiae in various tissues in the treated animals, as compared to the detection of few or no organisms in the same tissues from non-irradiated similarly infected control animals.

The Q fever antibody response was determined in the non-irradiated and irradiated infected animals. This antibody was shown to vary markedly in titer with animal species, irradiation dosage and antibody type. Phase I and II complement fixing (CF) antibody titers were depressed in sera from irradiated guinea pigs at one week after irradiation, but increased significantly in animals receiving the high irradiation dose. A negative antibody phase occurred in the low dose-irradiated animals by four weeks. Phase I capillary tube agglutination (CTA) antibody titers increased in high and low irradiation dosage groups by one week, then subsequently decreased in the low dosage irradiated guinea pigs. The antibody titers varied less in the tested white mice than in the guinea pigs, but the same general trends of negative antibody phase and titer increase were noted. Little titer variation occurred in deer mice sera, although a slight depression of CF antibody occurred in the irradiated animals one to two weeks after treatment.

Normal control guinea pigs and white mice housed as cage mates with infected, irradiated animals developed Q fever antibody titers in their sera, indicating a transmission of C. burnetii from the infected animals, presumably as a result of irradiation. No transmission could be observed in

deer mice and non-irradiated infected animals.

Injections of low or high concentrations of cortisone given daily for seven days induced a reactivation of C. burnetii infection in guinea pigs and white mice but not in deer mice infected three months previously. Similar methods to the x-irradiation studies were employed for this determination. The multiple cortisone injections had only a moderate effect upon the Q fever antibody response in the three animal species tested. A depression of antibody titer occurred in most animals at varying time intervals following cortisone injections. This was usually preceeded by a sudden increase in CF titer during the treatment. Reactivation of the Q fever infection by cortisone treatment resulted in a spread of the disease agent, as had occurred in the x-irradiation studies. Transmission apparently did not occur among treated deer mice or untreated infected animals.

A reactivation of Q fever infection occurred sporadically following parturition in guinea pigs and white mice previously infected with C. burnetii. Various organs and blood, urine and feces from these animals were shown to contain C. burnetii as soon as 8 days and as long as 20 days after birth of offspring. Tissue from partus born from the infection-reactivated parents were also often infected. Little Q fever antibody titer change occurred in either parents or progeny.

A secondary Q fever antibody response was studied in guinea pigs reinfected with C. burnetii three months after primary infection. A plot of the mean titers showed the antibody titers dropped slightly, then increased moderately after one to two weeks. The titers gradually decreased to, or slightly below, the original titer. Intraperitoneal, subcutaneous and intravenous routes of secondary injection were employed in separate experiments, with little difference noted in induced antibody titers.

RESEARCH PROPOSALS

1. The lack of a reproducible method of causing death in laboratory or wild animals due to a Coxiella burnetii infection has hampered many investigations with the organism. The determination of such a method is therefore of paramount importance. It is possible that intracerebral inoculation of various aged susceptible animals with C. burnetii, or the use of a resistance-lowering stress may prove effective.

2. Studies of the ingestion and cytopeptic action of immune and normal phagocytes upon C. burnetii should be initiated. An effective LD_{50} as described in (1) would be employed. Methods have recently been introduced for the purification of C. burnetii without loss of infectivity and for labeling the rickettsiae with P^{32}, which would be valuable in phagocytosis studies.

3. Some controversy exists regarding the relative protective capacity of Phase I and Phase II Q fever antibody. It is proposed that the effect of each upon phagocytosis, employing the methods described in (2) be investigated.

4. A secondary Q fever antibody response was investigated in the current thesis. Further studies of the secondary Q fever antibody response should be considered employing various dosages of C. burnetii, adjuvants, or multiple injections of the organism.

5. The present study has shown that injections of cortisone, an adrenocortical hormone, can result in the reactivation of latent Q fever infection. It is proposed that the effect of other types of hormones on latent Q fever infections also be investigated.

6. Studies were described in the present investigation concerning the effect of cortisone injection and whole body x-irradiation upon the Q fever antibody response in latently infected animals. It is proposed that studies of other factors in immunity such as phagocytic activity be carried out.

7. Since it has been shown that whole body x-irradiation can cause a reactivation of a latent infection with C. burnetii, it would be of interest to attempt to reverse such a reactivation by injection of a radiation protection chemical or one of the cellular or humoral resistance factors.

8. Only limited investigations of the effects of the natural serum factors such as complement, properdin, or lysozyme on rickettsiae have been reported. A detailed study of the effects of each upon a C. burnetii or other rickettsial infection should be considered.

9. No hemagglutination (HA) test has yet been devised for Q fever. Such a test, if developed, would be of value as an assay system for the disease agent. Various ions, temperatures, pH and types of red blood cells could be investigated in attempting to perfect the test.

10. Coxiella burnetii has been cultivated upon a variety of tissue culture cells. It is proposed that further investigations with tissue cultures be initiated in an attempt to produce an efficient assay system for the organism. Plaque formation would be the ideal indicator, if such could be shown to occur.